

CHAPTER 3: ECOLOGICAL EFFECTS OF PHOSPHORUS ENRICHMENT IN THE EVERGLADES

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SUMMARY

This chapter provides an update on data collection and analyses that will support the derivation of a numeric phosphorus (P) criterion for the Everglades. The purpose of this chapter is not to define what constitutes an ecological imbalance, nor is it to recommend how compliance with the numeric P criterion should be determined. Available information on the effects of P enrichment on the Everglades was reviewed in the 1999 Everglades Interim Report. This report focuses on major developments since the previous report, including: (1) data analyses to support the derivation of a numeric P standard for WCA-2A; (2) completion of preliminary analyses of District research and monitoring data from the Refuge; (3) regulatory and research developments in the central and southern Everglades, including the adoption of a P standard by the Miccosukee tribe and a District research program initiated in 1999.

WATER CONSERVATION AREA 2A: RESULTS OF CONTINUED DATA ANALYSIS

The most extensive ecological changes associated with P enrichment have occurred in WCA-2A. Initial regulatory efforts were focused on this area because of the large amount of available data for establishing background and enriched conditions. Data from gradient and transect studies were analyzed to document and evaluate the changes that occur in the various natural communities along the P enrichment gradient in WCA-2A.

Much of the P entering WCA-2A originates as Everglades Agricultural Area (EAA) runoff. This runoff flows through the S10 structures (S10s) along the northern levee. Canal inflows are elevated in P relative to the marsh interior, and have created an enrichment gradient extending 7-8 km downstream of the S10s. Annual mean total P (TP) concentrations in these inflow waters increased from 79 µg/L in 1978 to between 131 and 146 µg/L in the early 1980s and have since declined to 47 µg/L in 1997 and 62 µg/L in 1998. Marsh water column TP concentrations decline from >100 µg/L near the S10s to <10 µg/L in the interior marsh. In association with elevated water column P concentrations, P has accumulated in the marsh soils, and sediment TP concentrations range from <500 mg/kg in the marsh interior to >1,700 mg/kg near the canal inflows. Sediment TP concentrations between 500 and 600 mg/kg have frequently been used to indicate areas of enrichment in WCA-2A, and these levels extend as far as 6 to 8 km into the marsh downstream of the S10s.

Florida State narrative water quality criteria and the Everglades Forever Act specify that concentrations of P shall not “be altered so as to cause imbalance in natural populations of flora or fauna.” Therefore, the derivation of a numeric P criterion for WCA-2A requires that biological responses to P enrichment be understood. Flora and fauna occupying reference areas of WCA-2A are adapted to environmental conditions created by low P inputs. While different groups of organisms respond to P

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enrichment at varying rates, many of the major changes in natural flora and fauna appear to occur at similar levels of P enrichment.

Many important microbial processes, such as decomposition and nutrient cycling, are accelerated by P enrichment. For example, the activity of extracellular phosphatase enzymes used to sequester P decline relative to reference conditions at sampling stations within 7 km of the S10s. Phosphorus enrichment is associated with increased microbial biomass and respiration, and a shift towards anaerobic processes. Increased microbial activity in enriched areas of the marsh is associated with lower dissolved oxygen (DO) concentrations and more rapid nutrient cycling. Thus, microbial processes in P enriched soils may continue to mobilize P even after external inputs have been reduced.

Periphyton (submerged and floating mats of algae, bacteria and other microorganisms) is an abundant and ecologically important feature of the oligotrophic Everglades. Changes in the physical structure, nutrient content, and taxonomic composition of Everglades periphyton have been documented along marsh nutrient gradients and in controlled P enrichment studies in WCA-2A and other Everglades marshes. The abundant community of calcium-precipitating (i.e., calcareous) cyanobacteria and diatoms is replaced by a eutrophic assemblage of cyanobacteria and green algae in areas of the marsh within 7 km of the S10s where water column TP concentrations typically exceed background (TP >10 µg/L) levels and in controlled P dosing studies. Analyses of periphyton communities sampled along the P gradient between 1994 and 1998 showed a shift in dominance from taxa indicative of unpolluted, low P conditions towards those indicative of polluted, high P conditions at sampling stations between 7 and 8 km downstream of the S10s. Statistical analyses showed a significant change in the species composition of the periphyton community occurred between stations where water column P is elevated above background concentrations and reference stations, where water column P is main-

tained within the background range for this marsh. Controlled P dosing experiments have shown that many of these taxonomic changes are a direct result of P enrichment.

Historically, major vegetative habitats in the Everglades included sawgrass marshes, wet prairies, and sloughs. Although cattail is widely distributed in low abundance across the oligotrophic Everglades, available evidence indicates that cattail was not a common vegetation type in the predisturbance Everglades. Cattail stands have expanded in WCA-2A, downstream of the S10s, to form over 2,692 acres of nearly monospecific stands and an additional 13,678 acres of mixed cattail-sawgrass-slough habitat. Numerous studies have linked this shift from sawgrass, wet prairie, and slough communities to monospecific cattail stands to P enrichment. Statistical analysis of monitoring data from the nutrient gradient indicated a significant shift from dominant sawgrass and slough vegetation to cattail between approximately 6 and 8 km downstream of the S10s, where soil P concentrations generally exceeded 500 mg/kg. These vegetation changes result in increased rates of soil accretion and nutrient cycling, and reductions in the spatial extent of open-water areas, which are crucial habitats for native plants and animals and sources of DO.

Benthic macroinvertebrates represent an important trophic link between Everglades primary producers and higher animals, and are affected by changes in DO, food sources, and habitat structure caused by P enrichment. Studies conducted in WCA-2A using different methodologies showed no consistent effect of P enrichment on macroinvertebrate species richness or diversity. However, changes in taxonomic composition with increasing P enrichment, including a shift towards species able to tolerate the low oxygen conditions, have been documented in enriched areas. The most extensive database on macroinvertebrate community changes has been compiled by the District and the Florida Department of Environmental Protection (DEP), based on repeated sampling of all major vegetation habitats along the S10 gradient

between 1994 and 1998 using both qualitative and quantitative sampling methods. Environmental indices calculated based on the taxonomic composition of these samples indicated that conditions in enriched areas were altered compared with the marsh interior. Statistical analyses indicated that the area affected by these changes included stations <7 km downstream of the S10s.

Oligotrophic areas of the Everglades exhibit wide fluctuations in water column DO but generally maintain aerobic conditions throughout the diel cycle. By contrast, DO concentrations in P enriched areas are depressed compared to unenriched areas and exhibit little fluctuation throughout the diel cycle and protracted periods of anoxia. These differences appear to be due largely to declines in the abundance of periphyton and submerged aquatic vegetation, which contribute oxygen to the water, and increased sediment oxygen demand in enriched areas of the marsh. Diel oxygen changes measured downstream of the S10s between 1995 and 1998 showed that water column DO declined with P enrichment. Statistical analyses indicated that daily mean and minimum DO declined significantly at stations <7-8 km downstream compared with those in the marsh interior. A controlled field P dosing study conducted by the District provided supporting experimental evidence that these declines are a result of P enrichment and not some other environmental change.

Results of data analyses completed to date for WCA-2A show that a number of ecological changes (microbial activity, periphyton, macrophyte, and invertebrate populations, marsh dissolved oxygen concentrations) occur along phosphorus gradients in this marsh and that many of these changes occur at stations where water column and soil TP exceed background levels of 10 µg/L and 500 mg/kg, respectively.

LOXAHATCHEE NATIONAL WILDLIFE REFUGE: PRELIMINARY RESEARCH FINDINGS

The District initiated gradient and mesocosm studies in the Refuge in 1996 using methods similar to those in WCA-2A. Both marshes have received inputs of nutrient-enriched canal waters from the same sources. However, whereas WCA-2A is a mineral-rich marsh, surface waters of the Refuge interior have an extremely low mineral content. These differences in background conditions may affect the sensitivity of the marsh to P enrichment.

Transect Sampling

Sampling has been conducted since April 1996 along an enrichment gradient in the Refuge to document chemical and biological changes. During this period, mean TP concentrations ranged between 51 and 56 µg/L at canal stations and declined to between 8 and 9 µg/L at marsh stations >2 km from the canal. Total dissolved P (TDP) and soluble reactive P (SRP) averaged 29-32 and 20-23 µg/L, respectively, in the canal and declined to 4-5 and 3-4 µg/L, respectively, in the marsh interior. Total nitrogen averaged 2.3-2.6 mg/L in the canal and declined to 1.0-1.3 mg/L at stations >2 km into the marsh. However, the TN:TP ratio suggested that P is the more important limiting nutrient in both the marsh and canal waters. Concentrations of major ions also declined with increasing distance from the canal, but at a slower rate than for either P or N. Water quality patterns along this enrichment gradient in the Refuge generally are similar to those south of the S10s in WCA-2A except that the gradient in the Refuge is shorter and much steeper, indicating that canal waters do not intrude as far into this rainfall-driven marsh.

The soil nutrient gradient in the Refuge also is not as extensive as observed in WCA-2A, and signs of soil P enrichment (>500 mg/kg) were confined to stations within 2 km of the canal. The relationship between soil and water column TP concentrations in the Refuge was similar to that in

WCA-2A, as soil TP increased rapidly at water column TP ranging between 10-20 $\mu\text{g/L}$ and leveled off at concentrations $>40 \mu\text{g/L}$. Porewater TDP and TDKN declined from concentrations $>250 \mu\text{g/L}$ and $>5.9 \text{ mg/L}$, respectively, within 1 km of the canal to $<10 \mu\text{g/L}$ and $<1.8 \text{ mg/L}$, respectively, in the marsh interior. Declines in most other nutrients, metals, and pH also were observed within the first 3 km of the canal.

Hydrolabs™ were deployed for multiple diel cycles on 3 occasions (June 1997, October 1997, February 1998) at marsh stations to measure diel fluctuations in water column DO. Mean DO declined from 2-8 mg/L in the marsh interior to $<2 \text{ mg/L}$ near the canal, and the frequency and duration of anoxia increased near the canal. As in WCA-2A, rates of decline in water column DO were greatest in areas of the marsh where water column TP increased from 10 to 20 $\mu\text{g/L}$.

The rate of microbial processes increased with increasing P enrichment. Decomposition rates, measured as the rate of tensile strength loss of standardized cellulose material, were similar in the Refuge and WCA-2A, and increased roughly two-fold with increasing enrichment along both gradients.

Standardized laboratory algal assays designed to measure the nature and extent of nutrient limitation in surface water samples indicated that P was the principal nutrient limiting algal growth throughout the marsh, whereas a shift towards N limitation occurred periodically in canal waters. The algal growth potential (a measure of nutrient bioavailability) of water samples from the canal was more than 20-fold higher than those in the marsh interior, and was 2- to 3-fold higher at marsh stations near the canal than those in the interior. These findings were consistent with those in WCA-2A.

Periphytometers were deployed quarterly at each marsh station to measure periphyton biomass accumulation (as chlorophyll *a*) and taxonomic

composition. Biomass accumulation declined sharply with increasing distance from the canal, ranging between 5 and 25 mg chl *a*/m² at stations closest to the canal compared with $<1 \text{ mg chl } a/\text{m}^2$ at interior marsh stations. The relationship between biomass and water column TP was similar along enrichment gradients in the Refuge and WCA-2A and was strongest at water column TP concentrations between approximately 10 and 20 $\mu\text{g/L}$.

Periphyton abundance (density of natural units) decreased with increasing distance from the canal, ranging between 60,000 and 250,000 units/cm² at stations closest to the canal compared with densities $<60,000 \text{ units/cm}^2$ at interior stations. Cyanobacteria abundance tended to be greatest at stations near the canal while diatoms and chlorophytes were most abundant at stations further into the marsh. One group of chlorophytes, the desmids, comprised as much as 25 percent of the periphyton assemblage in the marsh interior, where waters were low in P and most other elements, and exhibited the greatest decline at stations near the canal. This decline may be related to the higher mineral content of marsh waters near the canal as well as an increase in P concentrations. Many of the oligotrophic taxa found in interior areas of the Refuge differ from those identified in WCA-2A as a result of differences in background water chemistry. However, periphyton taxa indicative of high P conditions tend to be similar in the two marshes and exhibit similar responses to increased P enrichment in both areas.

Changes in slough vegetation, measured using ground-based sampling methods, were similar along the enrichment gradients in the Refuge and WCA-2A. In both marshes, the dominant *Eleocharis* species were not found at transect stations where soil TP exceeded 500 mg/kg. Another common slough macrophyte, *Nymphaea odorata* was most abundant at stations where soil TP ranged between 500-700 mg/kg, while *Typha* spp. were found with increasing frequency in sloughs where soil TP exceeded 500 mg/kg.

Mesocosms

Mesocosms were installed in a slough in the interior of the Refuge in March 1996 and dosed weekly to increase P loading rates by 0 (controls) to 3.2 g P/m²/y. Data from the first two to three years of dosing (depending on the parameter measured) were available for analysis. Patterns of P accumulation generally were similar to those documented previously in mesocosms in WCA-2A. Phosphorus accumulated rapidly in the surficial flocculent soil layer, while P accumulation in deeper layers required months or years, dependent on the loading rate used. After more than two years of P loading in the Refuge, there was no observable increase in porewater P concentrations. However, porewater NH₄-N became elevated within four months at higher loads and then decreased over time in the highest loads. Soil activity, measured as cotton strip decomposition rates (see transects), increased rapidly in response to P loading. Decomposition rates were over two-fold higher at a P loading rate of 3.2 g/m²/yr compared to controls.

Increases in the nutrient content (carbon, nitrogen, and P) of the benthic (epipelon) and floating mats in Refuge mesocosms were similar to those observed in WCA-2A. Epipelon P content increased quickly in response to P loading and was elevated by two- to four-fold at loading rates of 1.6 and 3.2 g P/m²/yr compared with controls. The P content of the floating periphyton-*Utricularia* assemblage increased by as much as eight-fold higher at the highest loading rate compared with controls. Epipelon and floating mat N content also increased with P loading and was 15 and 30-50 percent higher than in controls at the highest loading rates.

Periphyton metabolism (gross primary productivity and respiration) increased in proportion to the P loading rate and, after three years of dosing, there was evidence of a progressive increase in these rates through time with continued P loading at higher rates. This pattern was generally similar to that observed in WCA-2A.

Tissue nutrient concentrations of the two dominant slough macrophytes, *Nymphaea odorata* and *Eleocharis elongata*, increased in response to higher loading rates by the second year of loading. The TP concentration of *Eleocharis* appeared to respond more rapidly and was elevated at loads as low as 0.8 g P/m²/y. By contrast, the TP concentration of *Nymphaea* was elevated only at the highest loading rate during the first two years of dosing. The TN concentration of *Eleocharis* was elevated at all loads compared to the control after two years, whereas that of *Nymphaea* was elevated only at the highest loading rate.

Data collection by the District in the Refuge is ongoing and, therefore, conclusions based on available information from 1996-1998 should be viewed as preliminary in nature. However, initial findings clearly show that the Refuge is an oligotrophic, P limited marsh that is sensitive to increased P inputs. Many chemical and biological changes documented in the Refuge are similar to those documented previously in WCA-2A, and preliminary analyses suggest that the sensitivity of the biota in the two marshes to P enrichment also is similar.

CENTRAL AND SOUTHERN EVERGLADES

In May 1999, the EPA approved the 10 µg/L water column quality standard adopted by the Miccosukee Tribe of Indians of Florida (Tribe, MTIF 1997). Citing peer-reviewed publications and technical reports, the EPA determined that the 10 µg/L standard was a “scientifically defensible value which is not overly protective” and will protect the Class III-A designated use. It also stated, however, that additional Everglades data are still being collected and if further studies show that 10 µg/L is not protective of Class III-A waters, then the Tribe should revise its standard as necessary. A loss of structure and function of natural Everglades ecosystem was considered contrary to the tribal Class III-A designated use of “preservation of native plants and animals.” Therefore, the EPA evaluated

the effects of P enrichment on various components of the Everglades ecosystem as described in McCormick et al., (1999). The widely documented sensitivity of native periphyton, macrophyte, and microbial communities to P enrichment was used by EPA to support the Miccosukee's proposed P standard.

During 1999, the District obtained funding from EPA to conduct gradient and experimental studies in WCA-3A and Taylor Slough (Everglades National Park). Both gradient (transects) sampling and mesocosm dosing experiments will commence in August 1999 and continue through August 2000, with initial results becoming available during 2000. Results will be compared with those from northern Everglades marshes (WCA-2A, the Refuge) to assess spatial variation in ecosystem sensitivity to P enrichment.

EXPERIMENTS TO UNDERSTAND MECHANISMS UNDERLYING VEGETATION CHANGES IN EVERGLADES

Field and greenhouse studies have been conducted to understand how increased P levels interact with other environmental changes to cause shifts in Everglades vegetation. These studies continue to provide information on how species such as sawgrass and cattail respond differentially to changes in P availability under different environmental conditions.

- A field reciprocal transplant experiment showed a close relationship between cattail expansion and P enrichment. Cattail plants transplanted to enriched sites grew more quickly than those transplanted to unenriched sites and produced new ramets that facilitated plant expansion.
- An experiment examining biomass allocation and spatial expansion of sawgrass and cattail in different P environments determined that plants of both species produced more ramets and total biomass in the enriched treatment.

However, while sawgrass ramets grew close together within the pots, cattail ramets spread widely outside of the pots, thus facilitating rapid expansion.

- An experiment conducted under controlled greenhouse conditions investigated the effects of P availability on growth, biomass allocation, and nutrient use efficiency in sawgrass and cattail. No clear relationship existed between P availability and the growth, biomass allocation, and nutrient use efficiency of sawgrass. In contrast, cattail growth and biomass allocation to photosynthetic (i.e., growth) tissues were positively related to P availability; however, cattail was unable to grow at extremely low P levels. These patterns were consistent with the finding that sawgrass had a greater ability to scavenge P at low concentrations while cattail had a greater capacity for P uptake at high concentrations.
- The effect of P enrichment on seed germination and seedling growth was measured by placing pots containing seeds of cattail and sawgrass in the Refuge mesocosms. Increased P loading did not affect seed germination of either species. However, the growth and survivorship of cattail seedlings increased at higher P loading rates, whereas P enrichment increased the growth, but not the survivorship, of sawgrass.
- The effects of water depth and P concentration on the recovery of sawgrass and cattail stands following moderate disturbances, such as a surface fire, were determined by clipping the leaves of plants of both species and then placing them in P enriched or unenriched tanks under saturated or flooded conditions. Leaf regrowth of both species was reduced under flooded conditions but was unaffected by P concentration. These preliminary findings suggest that water depth may be the more important factor determining the rate at which established vegetation stands recover from surface fires.

These experiments support the findings of previous research by indicating the importance of P enrichment in determining the competitive abilities of cattail and sawgrass in the Everglades.

CONTINUING EFFORTS

Research and monitoring efforts by the District and DEP have expanded to include all major regions of the Everglades. While this work continues to focus principally on open-water (wet prairie, slough) habitats, available information indicates

that these ecologically critical areas are especially sensitive to P enrichment. Therefore, numeric P standards that are protective of these habitats should be protective of the entire marsh. Continued data collection and analyses by other research groups working in specific regions of the Everglades should contribute additional information on the effects of P enrichment in different regions and habitats. A synthesis of this information has been initiated by DEP to support the determination of a numeric P criterion for the Everglades based on best available scientific information.

INTRODUCTION

Defining the “Ecological needs of the Everglades” requires consideration of several factors including hydrology, nutrients such as phosphorus (P), other water quality issues (e.g., mercury, pesticides), and the spatial extent of habitats required to support self-sustaining populations of native species. For the purposes of this Consolidated Report, ecological needs will be defined primarily in terms of P (this chapter) and hydrology (**Chapter 2**). These chapters, in turn, correspond to Project RAM-6 (Interpret Class III Phosphorus Criterion Research) and RAM 10 (Hydrologic Needs of the Ecosystem) of the State’s Everglades Program Implementation Program Management Plan (SFWMD and DEP, 1997). Other water quality issues are discussed in **Chapter 4** (Water Quality of the Everglades Protection Area) and **Chapter 7** (The Everglades Mercury Problem).

A previous report (McCormick et al., 1999) synthesized available scientific information to determine:

- Reference (predrainage) conditions for P in the Everglades;
- Types of ecological changes caused by P enrichment; and

- Critical P concentrations and loads that cause changes in flora or fauna.

Information contained in that 1999 report supported the following conclusions:

- Native Everglades flora and fauna developed historically under conditions of extreme P limitation.
- Canal inflows have become a major source of P to the Everglades, particularly in northern areas.
- Increased P inputs have caused many ecological changes including the replacement of sawgrass, slough, and wet prairie habitats by a cattail marsh, loss of the native periphyton community, declines in dissolved oxygen concentrations, and changes in invertebrate species composition.
- Many of these changes occur at water column P concentrations between 10 and 20 µg/L, which are not far in excess of background levels (≤10 µg/L).

In the Everglades Interim Report, the District reviewed available information on the effects of P enrichment in the Everglades (McCormick et al., 1999). This chapter updates data collection and

analysis efforts in support of the Everglades Forever Act (the Act) mandate to establish a numeric P criterion for the Everglades. The purpose of this chapter is not to define what constitutes an ecological imbalance, nor is it to recommend how compliance with the numeric P criterion should be determined. First, data collection and analyses being conducted by the District and the Florida DEP to support rulemaking in WCA-2A are described. Preliminary findings from a monitoring and research effort initiated by the District in the Loxahatchee National Wildlife Refuge (the Refuge) in 1996 are then presented. Finally, regulatory and research developments in the central and southern Everglades, including the adoption of a P standard by the Miccosukee tribe and a District research program initiated in 1999, are summarized.

As with the Interim Report, information provided in this chapter is based on: (1) manuscripts published in the peer-reviewed literature; (2) widely distributed reports containing otherwise unpublished findings; and (3) unpublished analyses based on publicly available data that have passed DEP QA/QC screens. This chapter is intended to update, but not duplicate, the companion chapter in the Everglades Interim Report (McCormick et al., 1999), which is cited where applicable to support analyses and conclusions presented here. Written requests for all available manuscripts, reports, and unpublished data were sent to all major research groups conducting research and monitoring in the Everglades and relevant information received has been included in this report. The findings and conclusions reported should be considered interim, pending the final results of ongoing studies by several research groups.

WATER CONSERVATION AREA 2A: RESULTS OF CONTINUED DATA ANALYSIS

Efforts have begun to evaluate available data on the ecological effects of P enrichment in the Everglades to support the derivation of a numeric water quality standard for this nutrient. The current timeline for these efforts is shown in **Table 3-1**. Initial analyses have focused on WCA-2A because of the large amount of available data for establishing background and enriched conditions in this marsh.

Table 3-1. Schedule for Environmental Regulation Commission briefings by DEP to propose P standards for major regions of Everglades

Region	Briefing Date
WCA-2	May 1999
Refuge	November 1999
WCA-3	May 2000
ENP	December 2000

Available data from P gradient studies were subjected to standard QA/QC screens by DEP, and data that passed these screens were used to document changes that occur in various ecological parameters along the P enrichment gradient in WCA-2A. Data collected from least affected areas in the marsh interior were used to establish baseline or reference biological conditions. The results of this evaluation were then used to establish locations along the P gradient and corresponding P concentrations where populations of flora and fauna have changed from the reference condition. This approach is consistent with current practices in aquatic impact assessment in general (e.g., Hughes, 1995) and in wetland mitigation and restoration (e.g., Brinson and Rheinhardt, 1996). Where currently available, results from various experimental studies have been used to support and corroborate the conclusions drawn from the gradient studies.

EVIDENCE FOR P ENRICHMENT

Sources of Enrichment

Canal waters originating from Everglades Agricultural Area (EAA) runoff are the primary source of water and P to WCA-2A. Most EAA runoff enters WCA-2A through the S10 structures (S10s) along the northern levee (**Figure 3-1**). Smaller volumes of agricultural drainage discharge into the southwest portion of WCA-2A through the S7 structure. Water entering WCA-2A through the S10s flows across this marsh in a southerly direction. By contrast, inputs through the S7 tend to move in a southeasterly direction along the perimeter levee and bypass the interior of WCA-2A before entering WCA-3A through the S-11 structures. Consequently, the most pronounced influence of canal water on WCA-2A occurs in the northeast portion of the marsh downstream of the S10s.

Drainage leaving the EAA is enriched with respect to nutrients, especially P, as compared to the naturally oligotrophic waters of the Everglades. Annual mean total P (TP) concentrations in these inflow waters increased from 79 $\mu\text{g/L}$ in 1978 to between 131 and 146 $\mu\text{g/L}$ in the early 1980s and have since declined to 47 $\mu\text{g/L}$ in 1997 (Smith & McCormick, in press) and 62 $\mu\text{g/L}$ in 1998 (unpublished District data). By contrast, TP concentrations in surface waters in the interior of WCA-2A typically average below 10 $\mu\text{g/L}$. Discharges of canal waters into this oligotrophic marsh have produced a nutrient gradient downstream of the S10s (and to a lesser extent adjacent to the S7). This gradient has been extensively documented and studied by numerous research groups including the District, Duke University Wetland Center (DUWC), University of Florida, Florida International University, as well as other public and private entities.

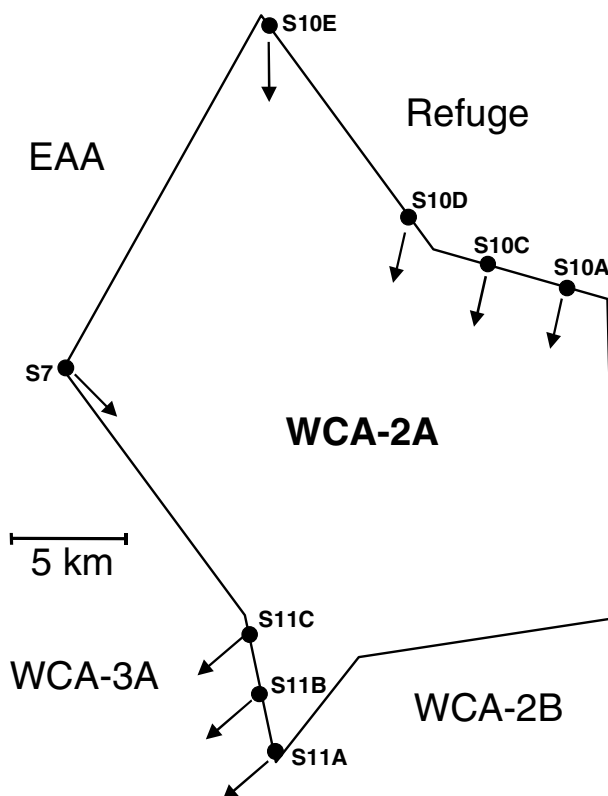


Figure 3-1. General flow patterns of water discharged through WCA-2A inflow and outflow structures.

Marsh Water Column P

Both the District and DUWC have monitored nutrient concentrations in north-eastern WCA-2A at permanent sampling stations extending from the S10s to as far as 14.5 km downstream in the interior marsh (**Figure 3-2**). A pronounced P gradient occurs downstream of the inflows with concentrations declining from $>100 \mu\text{g/L}$ in areas of the marsh near the inflows to $<10 \mu\text{g/L}$ in the interior marsh (**Figure 3-3**). Five interior ($>8 \text{ km}$ downstream of the S10s) stations monitored by the District provide the best indication of natural background chemical and biological conditions for WCA-2A (see Biological Effects following) and, thus, have been selected as reference stations. Oligotrophic conditions have been maintained at these stations between 1994 and 1998, with median water column TP during the period ranging between 7 and 9 $\mu\text{g/L}$ (**Figure 3-4A**). Occasional excursions of P above this background range at these sites may result from sample contamination and/or the

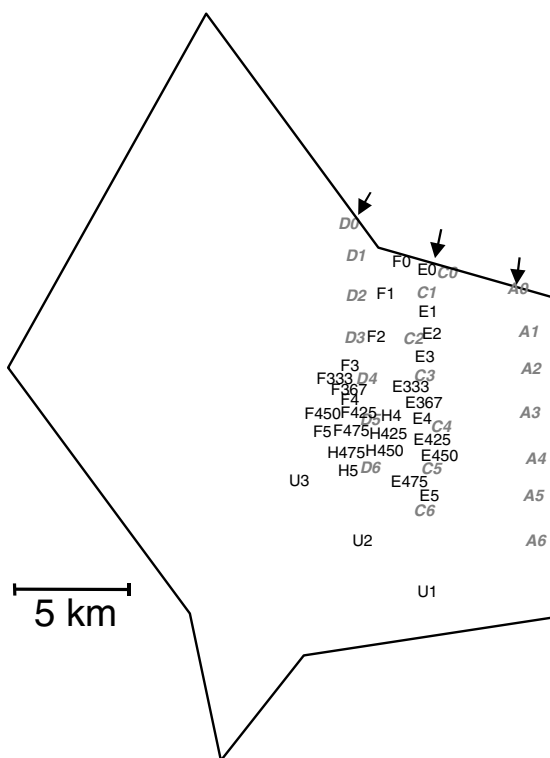


Figure 3-2. Location of DUWC (grey) and District (black) transect sampling stations.

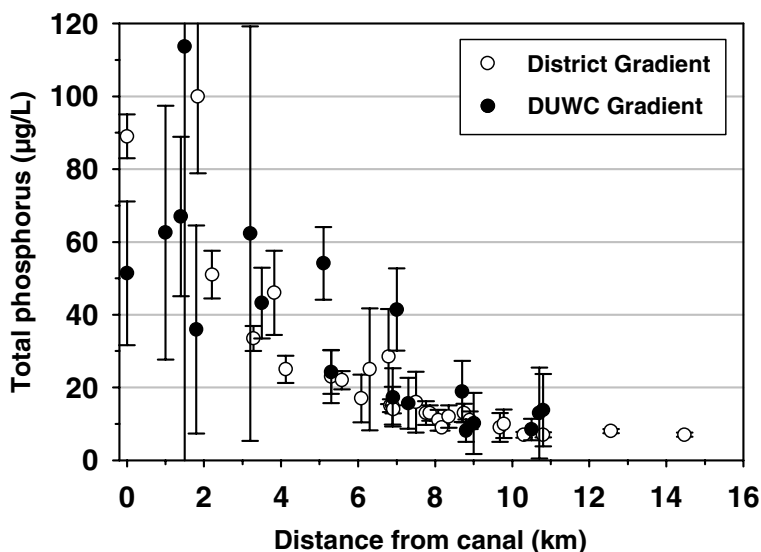


Figure 3-3. Water column TP (median $\pm 95\%$ confidence interval) along WCA-2A transect downstream of the S10 structures. DUWC sampling was conducted between January 1990 and November 1997, and District sampling was conducted between September 1994 and January 1998.

remobilization of P during marsh drying. Long-term data (1978-1998) are available for one of these stations (U3) and indicate that P concentrations in the reference area have been relatively stable over even longer time periods. Annual median TP concentrations at U3 have ranged between 5.0 and 25.0 $\mu\text{g/L}$ (**Figure 3-4B**). Highest concentrations occurred during the 1980s, possibly due to hydrologic changes caused by a modified water delivery schedule and by drought (Smith & McCormick, in press). However, despite periodic increases in TP during that period, the median TP concentration for this site during the decade of the 1980s was similar to that during the 1990s (9.7 and 7.0 $\mu\text{g/L}$, respectively).

Marsh Soils

Much of the P entering WCA-2A through the S10s has accumulated in the marsh soils. Available data on soil TP concentrations obtained from three previous surveys (DeBusk et al., 1994; Richardson et al., 1997; US EPA, 1998) and from ongoing District and DUWC monitoring were used to generate a soil TP contour map for WCA-2A (**Figure 3-5**). This map shows the existence and extent of the P enrichment gradient in the sediment in WCA-2A downstream of the S10 and S7 structures. Sediment TP concentrations range from <500 mg/kg in reference areas

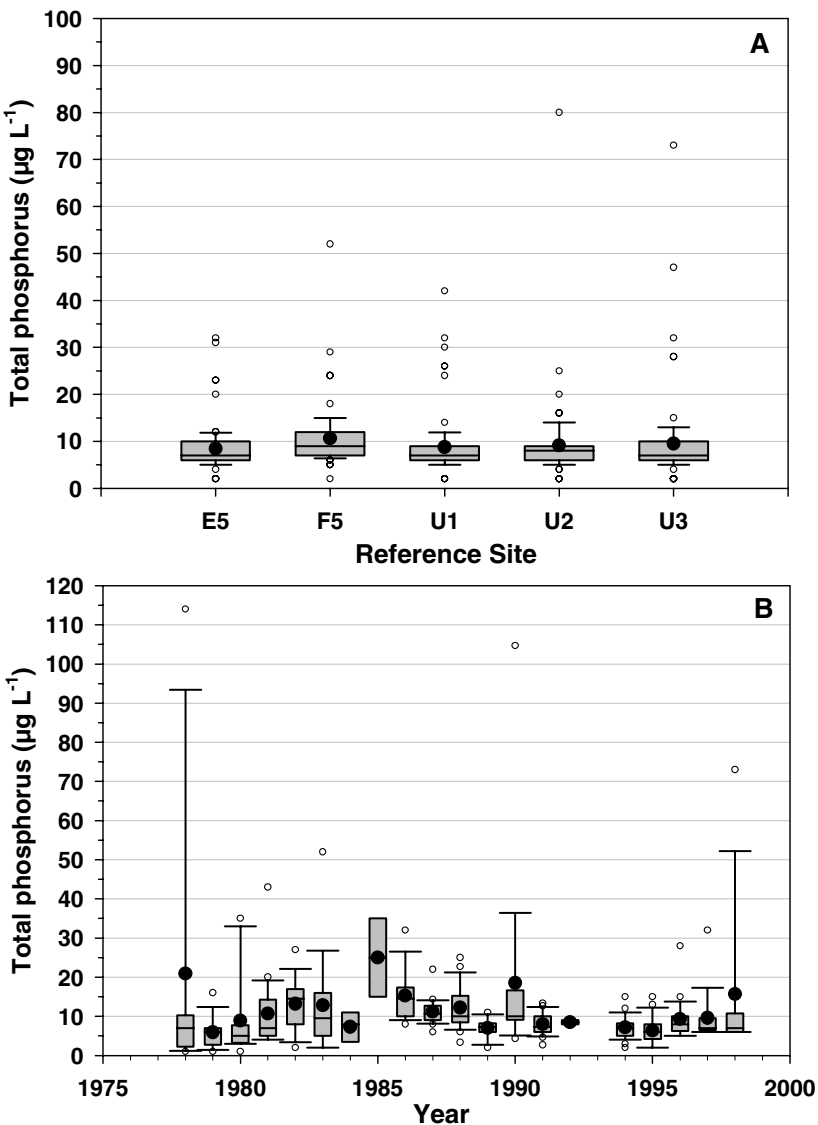


Figure 3-4. Box plots of water column TP concentrations in samples collected at **A.** District reference stations in WCA-2A between September 1994 and January 1998 and at **B.** District reference station U3 between 1978 and 1998. The top, mid-line, and bottom of each box represents the 75th, 50th (median), and 25th percentiles of data, respectively; the vertical lines represent the 10th and 90th percentiles; large closed circle is the arithmetic mean; small open circles are observations outside the 90th percentiles.

of the marsh interior to >1,700 mg/kg near the canal inflows. Soil TP concentrations between 500 and 600 mg/kg have been frequently used by researchers to indicate areas of enrichment in WCA-2A (Reddy et al., 1991; Craft & Richardson,

1993). Using the 500-600 mg/kg contour from **Figure 3-5**, the sediment P gradient in WCA-2A is estimated to extend as far as 6 to 8 km into the marsh downstream of the S10s.

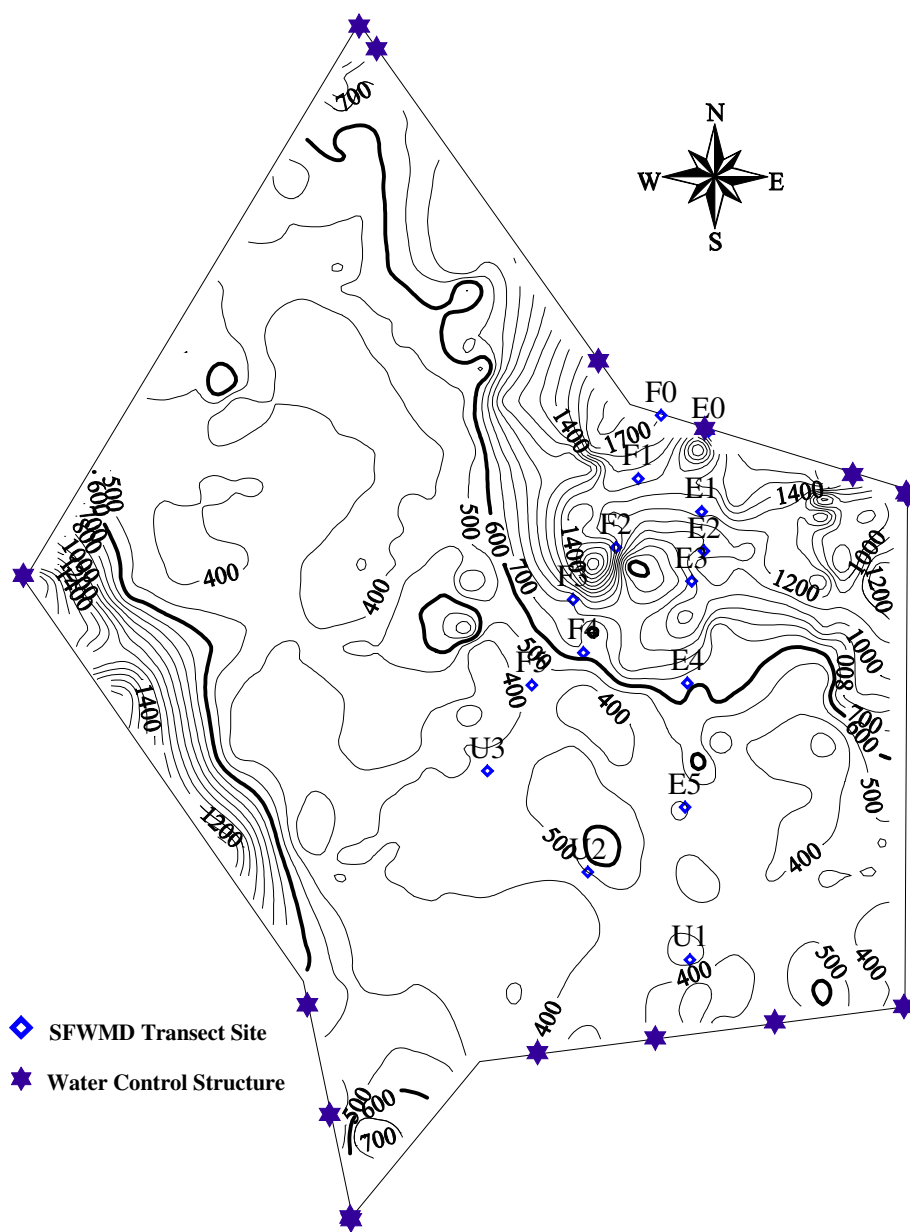


Figure 3-5. Map of sediment TP (mg/kg) contours in WCA-2A. See text for sources of data.

BIOLOGICAL EFFECTS OF PHOSPHORUS ENRICHMENT

Florida State narrative water quality criteria and the Everglades Forever Act specify that concentrations of P shall not “be altered so as to cause imbalance in natural populations of flora or fauna.” Therefore, the derivation of a numeric P criterion for WCA-2A requires that biological responses to P enrichment be understood. The flora and fauna occupying reference areas of WCA-2A are adapted to environmental conditions created by low P inputs and respond to P enrichment at varying rates. For example, relatively short-term P dosing experiments conducted by the District and DUWC have shown that microbial and periphyton communities respond to P enrichment within days or weeks, whereas rooted macrophytes and invertebrates may take several years to show a response. However, analyses of data collected along a nutrient gradient in WCA-2A, which have been exposed to elevated P concentrations and loads for roughly three decades, show that many of the major changes in natural flora and fauna occur at the same location along this gradient.

Microbial Processes

The microbial community controls key ecosystem processes such as decomposition and nutrient cycling. Phosphorus is the primary factor limiting microbial activity in oligotrophic areas of the Everglades and, therefore, many microbially mediated processes that affect water and sediment chemistry and plant productivity are accelerated by P enrichment.

Under P limited conditions, microbes respond quickly to increases in P availability by reducing the production of extracellular enzymes, phosphatase activity (PA), used to sequester P from the surrounding water and soil. Extremely low PA occurs near canal inflows in the Everglades, while activity is correlated strongly with P concentrations in areas of the marsh further downstream (Nearhoof, 1992; Richardson et al., 1997; USEPA, 1998; McCormick et al., 1999). In WCA-2A, for example, Richardson and Qian (in press) found that PA was nearly undetectable within 7 km of the S10s and increased as P concentrations declined further downstream (**Figure 3-6**).

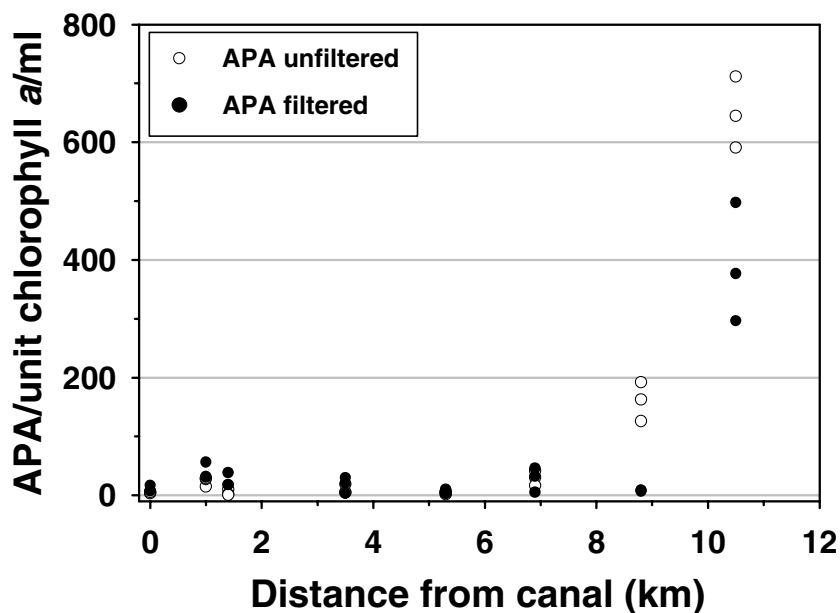


Figure 3-6. Alkaline phosphatase activity (APA) collected from DUWC transect stations in WCA-2A on August 15, 1995.

In WCA-2A, P enrichment is associated with increased microbial biomass and respiration and a shift in microbial community composition from aerobic and facultative anaerobes to obligate anaerobes (Reeder and Davis, 1983; Reddy et al., 1999). Similar shifts have been reported by researchers working in P enriched areas elsewhere in the Everglades (Bachoon & Jones, 1992; Amador & Jones, 1993). Amador and Jones (1993) examined the relationship between P and microbial activity experimentally by adding phosphate to Everglades Protection Area (EPA) peat soils of low (231 mg P/kg soil), intermediate (386 mg P/kg soil) and high (1,473 mg P/kg soil) P content. Phosphate addition stimulated sediment microbial respiration at low and intermediate P levels, but did not affect respiration rates in high P soils.

Phosphorus additions to Everglades marshes have been shown to accelerate rates of microbial decomposition (Maltby, 1985; Reddy et al., 1999). Increased soil decomposition rates are associated with lower dissolved oxygen (DO) concentrations and more rapid nutrient cycling (Maltby, 1985; Richardson et al., 1991; Reddy et al., 1999). Thus, microbial processes in P enriched soils may continue to mobilize P even after external inputs have been reduced.

Periphyton

Periphyton (submerged and floating mats of algae, bacteria and other microorganisms) is abundant throughout oligotrophic areas of the Everglades (Swift, 1981). Periphyton typically accounts for much of the vegetative biomass and primary productivity in open-water habitats such as sloughs and wet prairies, and is ecologically important as a habitat and food source for macroinvertebrates and small fish, as a source of oxygen for maintaining aerobic conditions in the water column, and in the process of soil formation in many areas (Gleason & Spackman, 1974; Wood & Maynard, 1974; Browder et al., 1982; Rader, 1994; McCormick et al., 1998).

The extremely low P concentrations (<0.05 percent) and extremely high N:P ratios (>60:1, w:w) of periphyton in interior areas of the Everglades are suggestive of strong P limitation (Swift & Nicholas, 1987; Grimshaw et al., 1993; McCormick et al., 1998). Significant changes in the physical structure, nutrient content, and taxonomic composition of Everglades periphyton have been documented along marsh nutrient gradients and in controlled P enrichment studies in WCA-2A and other Everglades marshes (Steward & Ornes, 1975 a, b; Swift, 1981; Swift, 1984; Swift & Nicholas, 1987; Flora et al., 1988; McCormick & O'Dell, 1996). These studies indicate that changes in periphyton abundance, productivity, and composition result both from direct effects of P on periphyton growth as well as from other P induced ecological changes (e.g., shading cause by increased growth and coverage of dense emergent macrophyte stands).

Oligotrophic, alkaline areas of the Everglades, such as WCA-2A, contain a characteristic periphyton community, consisting primarily of the calcium-precipitating (i.e., calcareous) cyanobacteria, *Scytonema* and *Schizothrix*, and a group of hard-water diatoms (e.g., *Mastogloia*) (Gleason & Spackman, 1974; Browder et al., 1981; Swift, 1981; Swift, 1984; Swift & Nicholas, 1987; McCormick et al., 1998). This periphyton assemblage rapidly removes excess P from the surrounding water and, thus, is quite sensitive to increased P inputs. Increases in periphyton P concentration caused by P enrichment alters both the physiology and physical structure of this community and eventually results in its replacement by a eutrophic assemblage of cyanobacteria and green algae in P enriched areas of the marsh (Swift & Nicholas, 1987; McCormick & O'Dell, 1996). McCormick and O'Dell (1996) documented the loss of the calcareous periphyton community at District sampling stations along the nutrient gradient downstream of the S10s (**Figure 3-7**) where water column TP concentrations exceeded background (TP >10 µg/L) levels. These investigators also documented this same change when controlled P additions were conducted in oligotrophic areas of the marsh.

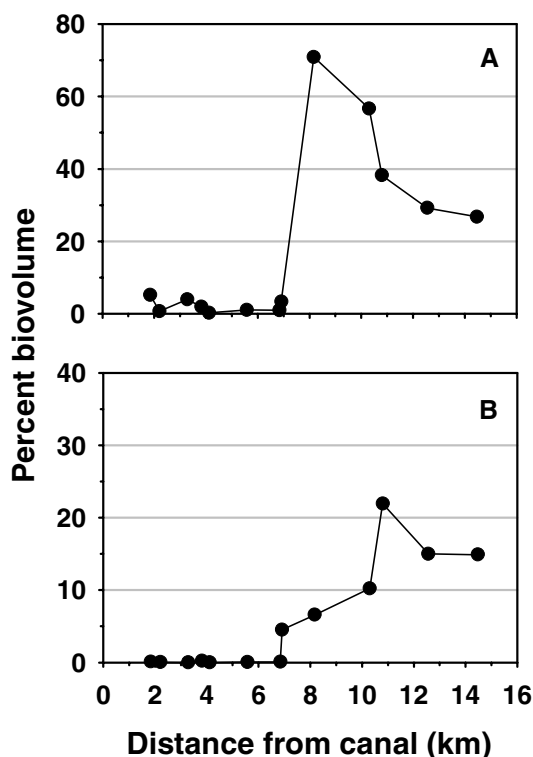


Figure 3-7. Percent biovolume of **A.** calcareous periphyton (*Scytonema*, *Schizothrix*) and **B.** sensitive diatom taxa in samples of periphyton floating mats collected at District transect stations in WCA-2A on December 5, 1995.

Ground-based surveys conducted by the District and DEP along this same gradient during February 1999 confirmed that the calcareous periphyton mat was absent from stations <7 km downstream of S10 inflows, where water column P concentrations consistently exceeded background levels. Similarly, controlled P enrichment of flume dosing channels in WCA-2A (Richardson et al., 1997) resulted in the loss of the calcareous community when bioavailable P (i.e., soluble reactive P (SRP)) was at least 5 $\mu\text{g/L}$ above background concentrations, which averaged near 4 $\mu\text{g/L}$ (McCormick and Stevenson, 1998).

A more extensive investigation conducted by the District between 1994 and 1998 involved the collection of periphytometer (artificial substrate)

samples during numerous monitoring events along the same gradient in WCA-2A. To assist in interpreting the taxonomic data, a list of pollution sensitive and tolerant taxa found in these samples was assembled based on a search of the available literature from the Everglades and elsewhere (Palmer, 1969; Lowe, 1974; Lange-Bertalot, 1979; Whitmore, 1989; Adamus, 1990; Romie, 1990; Bahls, 1992; McCormick & O'Dell, 1996; Richardson et al., 1997; L. Metzmeier, Kentucky Division of Water, pers. comm. (Table 3-2).

Sensitive taxa are absent near the canal inflows and exhibited the largest increase in abundance at a distance between 6.8 and 7.8 km from the canal (Figure 3-8). By contrast, tolerant taxa were relatively abundant near the canal and declined abruptly at a distance between 6.8 and 7.8 km from the canal inflows (Figure 3-8). Nearly identical patterns were found when the analysis was limited to a subset of these taxa, which had been identified as indicative of low and high P availability based on experimental dosing studies in WCA-2A. These analyses indicate that increases in P concentrations along the gradient were an important factor causing change in periphyton taxonomic composition.

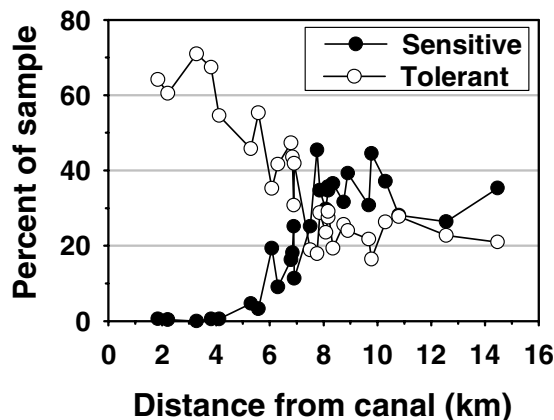


Figure 3-8. Percent abundance (natural unit densities) of pollution sensitive and pollution tolerant taxa in periphytometer samples collected at District transect stations in WCA-2A between April 1994 and June 1998. See Table 3-2 for literature sources used to select these indicator taxa.

Table 3-2. Periphyton taxa determined to be pollution-sensitive and pollution tolerant from the literature and phosphorus-sensitive based on data from District mesocosms in WCA-2A. See text for literature citations.

Pollution-sensitive	Pollution-tolerant	Phosphorus-sensitive
<i>Achnanthes exigua</i>	<i>Anabaena</i> spp.	<i>Anabaena subcylindrica</i>
<i>Achnanthes hustedtii</i>	<i>Cosmarium</i> spp.	<i>Anomoeoneis vitrea</i>
<i>Achnanthes linearis</i>	<i>Gomphonema parvulum</i>	<i>Cymbella lunata</i>
<i>Achnanthes microcephala</i>	<i>Lyngbya</i> spp.	<i>Cymbella minuta</i>
<i>Achnanthes minutissima</i>	<i>Navicula minima</i>	<i>Mastogloia smithii</i>
<i>Amphora ovalis</i>	<i>Navicula viridula</i>	<i>Synechococcus cedrorum</i>
<i>Anomoeoneis serians</i>	<i>Nitzschia amphibia</i>	
<i>Anomoeoneis vitrea</i>	<i>Nitzschia frustulum</i>	
<i>Cymbella lunata</i>	<i>Nitzschia palea</i>	
<i>Cymbella microcephala</i>	<i>Oscillatoria</i> spp.	
<i>Cymbella minuta</i>	<i>Rhopalodia gibba</i>	
<i>Mastogloia smithii</i>	<i>Scenedesmus</i> spp.	
<i>Navicula radiosa</i>		
<i>Synedra rumpens</i>		

Multivariate (cluster) analyses were utilized to assess similarity in periphyton taxonomic composition among District sampling stations and to determine which stations, if any, could be grouped with respect to the periphyton species found. Analyses performed on data collected from 13 stations between 1994 and 1998 showed two distinct groupings of stations: (1) a reference group including interior stations E5, F5, U1, U2, and U3; and (2) enriched stations near the canal (E1, E2, E3, F1, F2, and F3) (**Figure 3-9**). Stations E4 and F4 appeared to be transitional stations and clustered with the enriched group at a higher level. Similar results were obtained regardless of whether analyses were based on all taxa, common taxa only, or an even smaller subset of indicator taxa. As for analyses of indicator taxa, these cluster analyses indicate a substantial change in the species composition of the periphyton community between stations where water column P is elevated above background concentrations and reference stations,

where water column P is maintained within the background range for this marsh.

Change-point analysis (Niu et al., 1999) was used to evaluate statistically where a significant shift in the taxonomic composition of the periphyton samples described above occurred along the P gradient. To test for a change-point in the gradient, an iterative process is used. The process involves ordering the points along a gradient then comparing means above and below a certain point using a t statistic. The largest difference, if significant, is associated with the change-point. The test involves an adjusted critical value to account for multiple testing. The process may be used to find single as well as multiple change-points.

Change-point analysis indicated that both pollution-sensitive (i.e., determined from the broader literature) and P sensitive (i.e., determined from experimental dosing studies in WCA-2A) taxa declined relative to the reference condition at a dis-

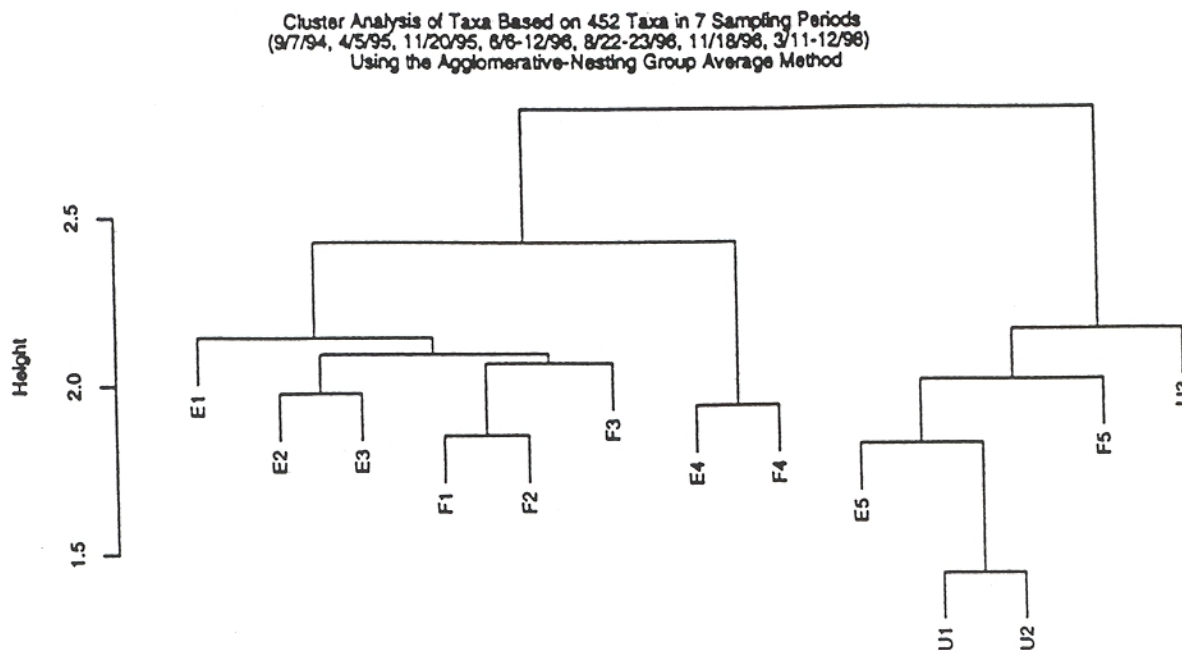


Figure 3-9. Results of cluster analysis (agglomerative-nesting group averaging method) of periphyton assemblages sampled at District transect stations in WCA-2A during seven sampling periods when all stations were sampled (September 1994, April 1995, November 1995, June 1996, August 1996, November 1996, and March 1998). Clustering is based on percent abundance of each taxon. The height of linkage shows degree of dissimilarity in taxonomic composition between stations.

tance of 7.5 km from the canal inflows. Similarly, results based on percent-tolerant taxa show an initial change-point occurs at a distance approximately 7 km from the canal. These results further support the conclusion that changes in the taxonomic composition of the periphyton community relative to reference conditions occurs at stations <7 km from canal inflows.

Both the District and DUWC have collected periphyton data from controlled P enrichment studies conducted in the oligotrophic interior of WCA-2A. While the relatively short-term nature of these studies cannot completely duplicate the long-term P enrichment that has occurred in the marsh, findings from these experiments provide data with

which to determine whether P enrichment is the primary factor contributing to periphyton (and other biological) changes along marsh nutrient gradients. For example, as documented along the marsh P gradient, P enrichment in District mesocosms caused the loss of the calcareous periphyton mat. Changes in periphyton taxonomic composition observed in P enriched mesocosms also corresponded to those documented along the marsh gradient. Some of these same changes have been documented in a flume dosing study conducted by the DUWC. However, the utility of the data obtained from the DUWC flume studies is limited because of the excessively high degree of observed variability in many response parameters.

Macrophytes

Native macrophyte vegetation in Everglades marshes is dominated by species adapted to low P concentrations, seasonal patterns of wetting and drying, and periodic natural disturbances such as fire, drought, and occasional freezes (Duever et al., 1994; Davis, 1943; Steward & Ornes, 1983; Parker, 1974). Historically, major habitats included sawgrass marshes, wet prairies, and aquatic sloughs (Loveless, 1959; Gunderson, 1994; also see **Chapter 2**). The characteristics of each of these habitats are described in McCormick et al., (1999). Although cattail is widely distributed in low abundance across the oligotrophic Everglades, available historical and paleoecological evidence indicates that cattail was not a common vegetation type in the predisturbance Everglades (Davis, 1946; Cohen & Spackman, 1984; Wieland, 1981).

Several studies have indicated that Everglades natural vegetation patterns are being altered by P enrichment. In particular, a rapid increase in the spatial distribution of cattail has been documented in enriched areas of the Everglades in recent decades. As of 1995, a monospecific cattail stand (>90 percent coverage) of approximately 1089 hectares (2,692 acres) existed downstream of the S10s, and an additional 5535 hectares (13,678 acres) formerly composed of primarily sawgrass and slough communities had been invaded by cattail (Rutchev and Vilchek, 1999). Numerous studies have found a link between increased P enrichment and a shift from sawgrass, wet prairie, and slough habitats to monospecific stands of cattail (Davis, 1991; Urban et al., 1993; Richardson et al., 1997; Miao & Sklar, 1998). Vegetation and soil mapping have shown that the areas where cattails have invaded closely follow the areas where sediment P concentration exceeds 500-600 mg/kg,

which is generally thought to be indicative of P enrichment in WCA-2A.

Recent studies conducted by DUWC and the District along the nutrient gradient downstream of the S10s in WCA-2A showed an increase in the abundance of cattail and exotic species such as water lettuce (*Pistia stratiotes*) and water hyacinth (*Eichornia crassipes*) and a decline in sawgrass and slough taxa (e.g., *Eleocharis*, *Nymphaea*, and *Utricularia*) with P enrichment, as shown in **Figures 3-10 and 3-11** (Vaithiyanathan et al., 1995; Richardson et al., 1997; McCormick et al., 1999). Field experiments have confirmed the relationship between these vegetation changes and P enrichment (Craft et al., 1995; Miao & DeBusk, 1999). Statistical (i.e., change-point) analysis applied to District data (**Figure 3-11**) indicated a significant shift from dominant sawgrass and slough vegetation to cattail between approximately 6 and 8 km downstream of the S10s, where sediment P concentrations exceeded 500-600 mg/kg. Vaithiyanathan and Richardson (1999) collected semi-quantitative data on plant distributions along this same gradient and found similar changes at sediment TP concentrations >700 mg/kg.

The vegetation changes just described also correspond to a reduction in the spatial extent of open-water areas, which are crucial habitats for the growth of floating and submerged aquatic vegetation and periphyton mats, important sources of DO for the marsh, and key foraging habitats for wading bird populations. Furthermore, increases in the coverage of cattail affects the nature and rate of peat accretion, which in turn affect rates of nutrient accumulation and cycling (Davis, 1991; Koch & Rawlik, 1993). Thus, vegetation changes caused by P enrichment have far-reaching consequences for all trophic levels.

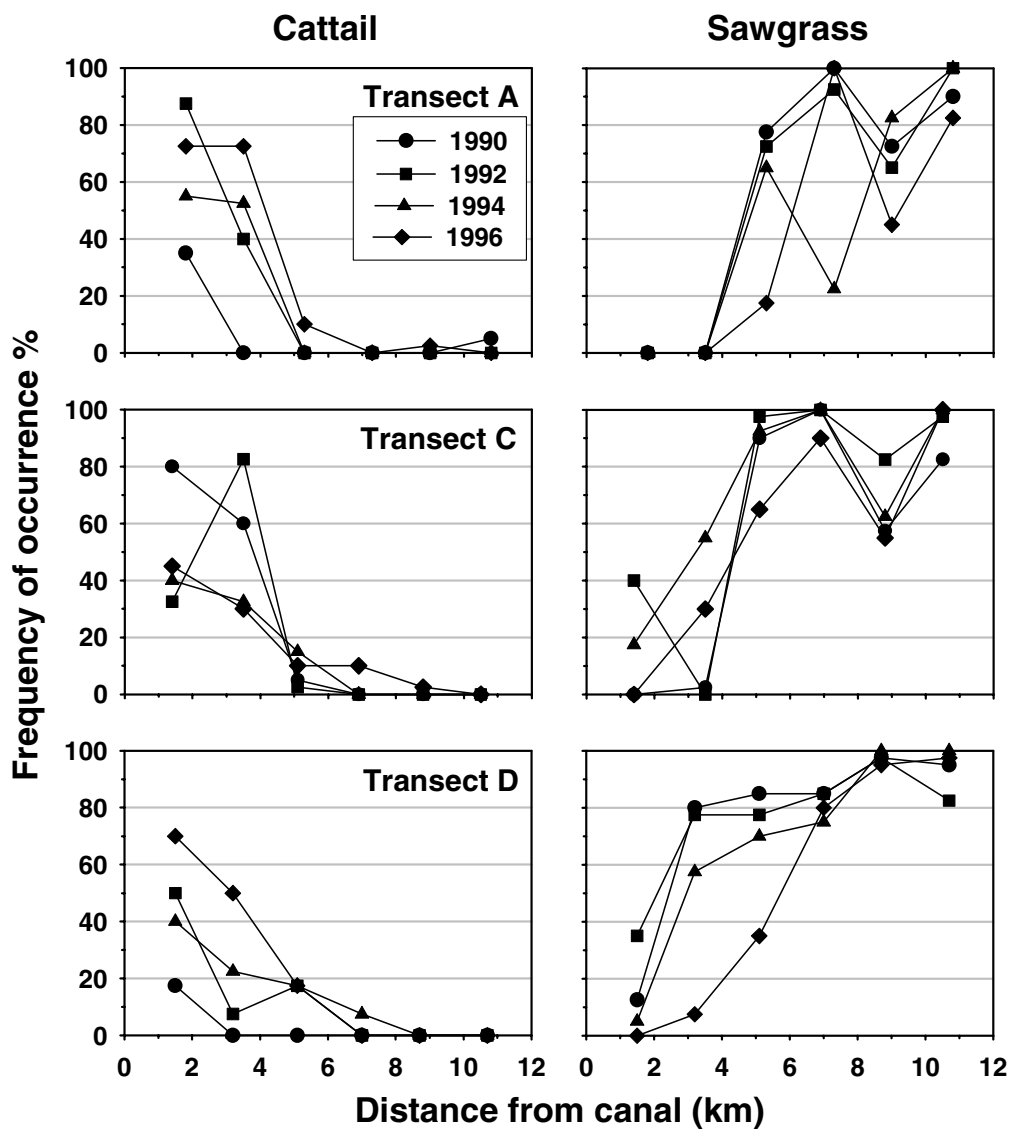


Figure 3-10. Frequency of *Typha* and *Cladium* occurrence in line-point transect sampling conducted in macrophyte stands at DUWC transect stations in WCA-2A during four sampling events between 1990 and 1996 (August 1990, May 1992, June 1994, and September 1996).

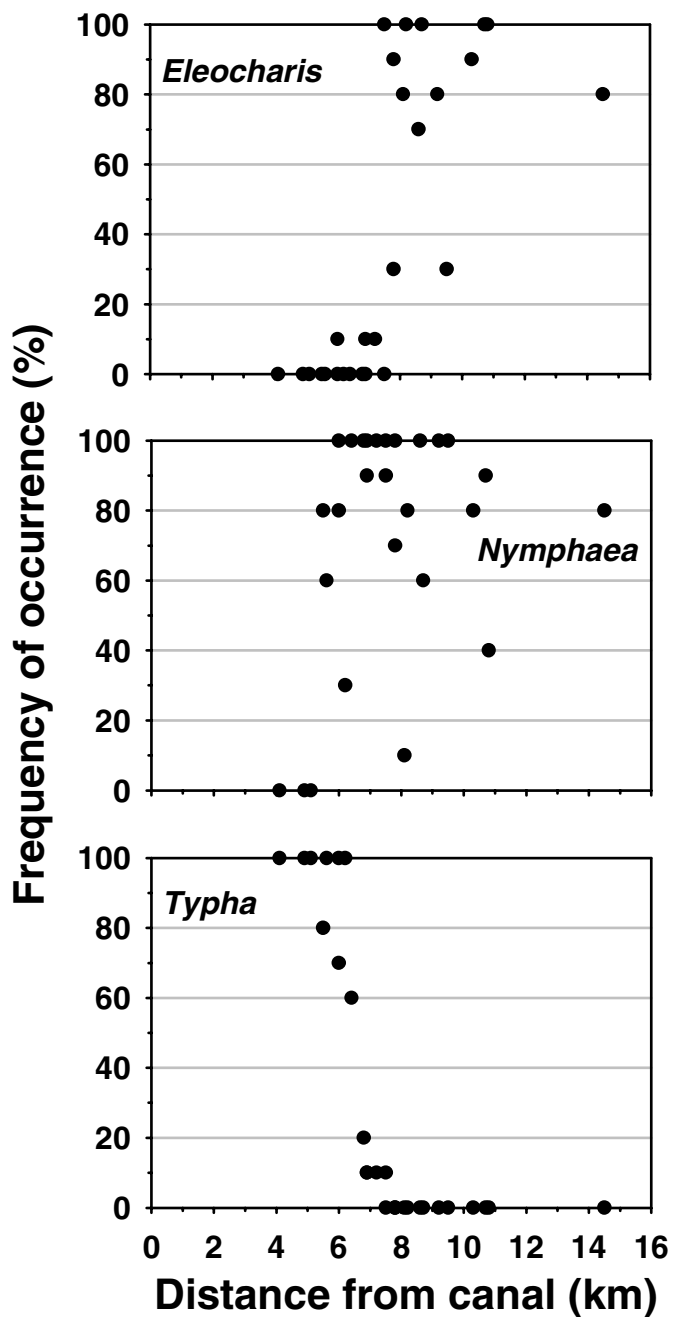


Figure 3-11. Frequency of occurrence of *Eleocharis*, *Nymphaea*, and *Typha* in 1-m² quadrants in sloughs at 27 District transect stations in WCA-2A based on sampling conducted during April 1997.

Macroinvertebrates

Benthic macroinvertebrates are the most commonly used group of organisms in water quality assessment and represent an important trophic link between Everglades primary producers and higher animals (e.g., fish, birds). Macroinvertebrates do not respond directly to P enrichment, but are affected by changes in DO, food sources, and habitat structure caused by such enrichment.

An early study of macroinvertebrate communities in the northern Everglades (Terczak, 1980) found that enriched stations had lower taxon richness and a distinct taxonomic composition compared with unenriched locations. By contrast, sampling conducted by DUWC downstream of the S10s (Rader & Richardson, 1994) showed an increase in macroinvertebrate taxon richness with increasing enrichment, largely as a result of an increase in the number of coleopteran (beetle) taxa. However, sampling was limited to open-water habitats and, thus, did not account for the considerably greater coverage of emergent macrophyte (i.e., cattail) stands – habitats that characteristically have low invertebrate diversity – in enriched areas.

To account for the effects of enrichment-related vegetation changes on the macroinvertebrate community, the District and DEP repeatedly sampled all major vegetation habitats along the S10 gradient between 1994 and 1998 using both qualitative (1994-1995) and quantitative (1996-1998) sampling methods. Taxonomic information generated from this sampling was used to calculate biological impact indices that are routinely used by DEP in bioassessments of Florida streams and lakes. These two indices assign points to stream- and lake-dwelling macroinvertebrate taxa, respectively, based on their sensitivity to pollution. Higher scores are considered to indicate better health for a stream or lake. No index of this type has been developed for Florida wetlands, but these two indices could be applied to the Everglades data as many of the taxa found in the Everglades overlap those found in other Florida aquatic ecosystems. Index scores increased with distance from

the S10s, indicating impacted conditions near the inflows and improving conditions farther downstream (Figure 3-12). change-point analysis indicated statistically significant increases (i.e., improvements) in index scores at stations 7-8 km downstream of the S10s.

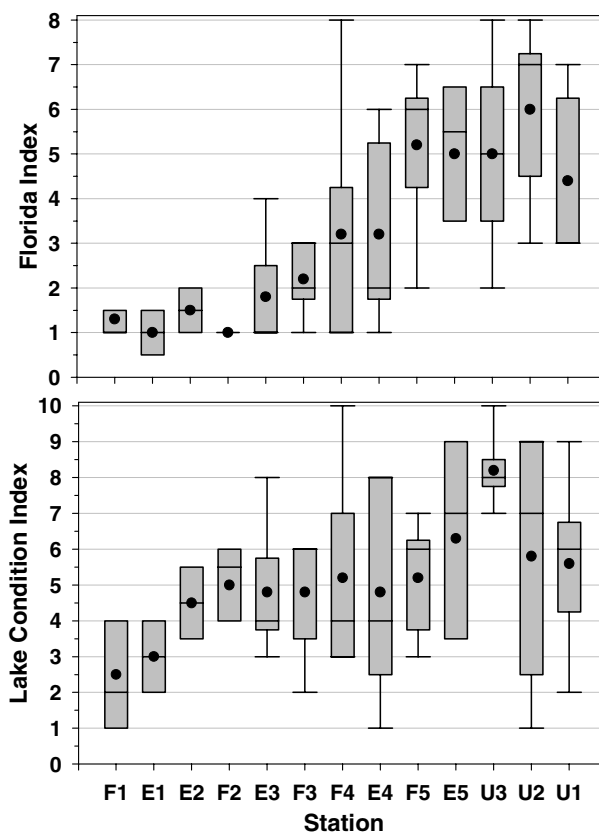


Figure 3-12. Changes in environmental indices (Florida Index, Lake Condition Index) based on macroinvertebrate taxonomic composition in qualitative dip net samples collected quarterly at District transect stations in WCA-2A between September 1994 and November 1995 (n=4 or 5 for each station). Higher index values reflect more pristine conditions and declines indicate increasingly impacted conditions.

As with periphyton community composition, cluster analyses performed on the entire macroinvertebrate community showed that stations E5, F5, U1, U2, and U3 clustered together while one or more separate clusters were generated for enriched stations (E1, E2, E3, E4, F1, F2, F3, and F4)

(Figure 3-13). These results agree with changes in impact indices by showing that stations <7 km downstream of the S10s have distinct invertebrate communities from those in reference areas of WCA-2A.

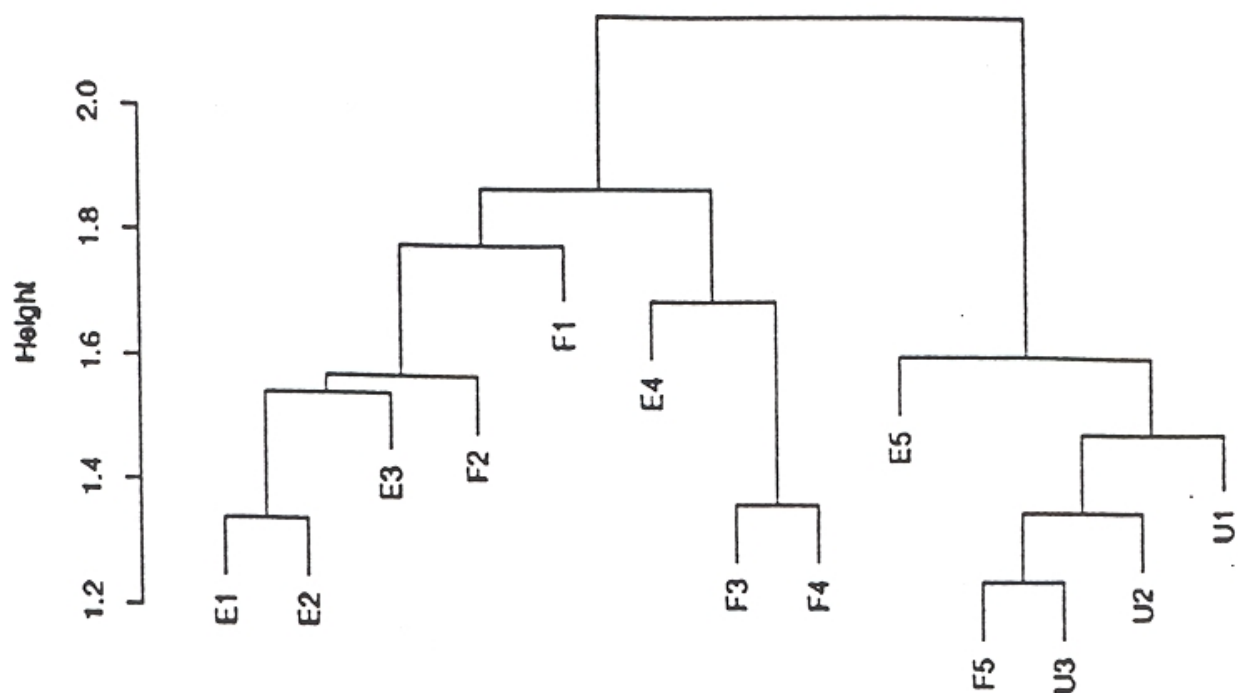


Figure 3-13. Results of cluster analysis (agglomerative-nesting group averaging method) of macroinvertebrate assemblages in qualitative dip net samples collected at District transect stations in WCA-2A during three sampling periods when all stations were sampled (September 1994, February 1995, and November 1995). Clustering is based on the percent abundance of each taxon. The height of the linkage denotes the degree of dissimilarity in taxonomic composition between stations.

Higher Trophic Levels

The amount of information on the response of larger predators (e.g., fish, birds) to P enrichment in the Everglades is small compared with that for periphyton, macrophytes, and invertebrates. Available information on this topic was reviewed in the Everglades Interim Report (McCormick et al., 1999). Fish, birds, and other top predators do not respond directly to increased P availability, but can be affected indirectly as a result of changes in food availability and the physical and chemical environment of the habitat. These environmental changes have been shown to affect both the abundance and species composition of higher trophic levels as described below.

Fertilization of aquatic systems with a limiting nutrient, such as P, invariably stimulates ecosystem productivity, resulting in an increase in both plant and animal biomass (Cooke et al., 1993). Turner and co-workers (1999) found a similar pattern in WCA-2A and elsewhere in the Everglades, where the standing crop of both invertebrates and fish were higher in habitats enriched by canal P inputs than in unenriched areas. These authors noted that the low standing crop of these consumers in unenriched areas is a distinctive feature of the natural Everglades and is lost upon enrichment.

Factors related to nutrient enrichment that are known to affect wetland bird populations include: (1) changes in prey density; and (2) changes in habitat structure, particularly the spatial extent and density of emergent macrophytes (Weller 1994). In nutrient-poor ecosystems such as the Everglades, food availability is an important factor affecting wading bird reproduction (Kushlan 1978, Frederick and Collopy 1989, Frederick and Spalding 1994). Many bird species avoid densely vegetated habitats such as cattail and sawgrass stands (Collopy and Jelks, 1987; Hoffman et al., 1994; Weller, 1994; Chavez-Ramirez and Slack, 1995). Thus, increases in the coverage of emergent vegetation as occurs along the WCA-2A gradient would be expected to negatively affect wading bird foraging activity. However, other wetland bird species (e.g.,

Least bitterns) can tolerate densely vegetated habitats (Frederick et al., 1990; Weller, 1994) and thus may be abundant in cattail stands. These species are able to forage effectively in densely vegetated habitats and, therefore, may respond positively to increases in prey density. Work is currently under way in WCA-2A to examine changes in bird density and species composition in response to nutrient-related habitat changes and will be discussed in later reports.

Water Column Dissolved Oxygen

Since the periphyton community appears to play an important role in maintaining the natural DO regime in the Everglades and has been shown to be among the first affected by P enrichment, DO may be a sensitive indicator of the biological status of the system. Dissolved oxygen concentrations in wetland environments such as the Everglades routinely fall below the Florida Class III standard (5 mg/L) on a daily basis and exhibit a wide diurnal fluctuation due to natural processes of photosynthesis and respiration. These variations represent the normal variability typical of this ecosystem (McCormick et al., 1997). However, despite such fluctuations, aerobic conditions are commonly maintained throughout the diel cycle in unenriched areas of the Everglades. By contrast, DO in nutrient enriched areas are depressed relative to the unenriched condition and exhibit little fluctuation throughout the diel cycle and protracted periods of anoxia (Belanger et al., 1989; McCormick & Laing, in review).

McCormick and Laing (in review) summarized the biological and chemical processes controlling water column DO dynamics. In oligotrophic sloughs, where light penetration to the water is high, high rates of photosynthesis by periphyton and submerged aquatic vegetation contribute large amounts of oxygen to the water during daylight hours. At night, periphyton respiration and sediment oxygen demand (SOD) cause a decline in water column DO concentrations. Oxygen production in unenriched sloughs exceeds respiration during the diel cycle and, therefore, generally provides

sufficient oxygen for maintaining aerobic conditions within the marsh. Increased growth and coverage of emergent macrophytes caused by P enrichment results in increased shading of the water column, which inhibits submerged photosynthesis, and an increase in the production of nutrient-rich detritus, which stimulates SOD.

Diel oxygen changes were measured by the District at 13 permanent transect stations downstream of the S10s between 1995 and 1998 (McCormick & Laing, in review). Measurements were performed in similar habitats (at the edge of stands of emergent vegetation) at all stations. Oxygen concentrations at stations <7 km from canal inflows generally were quite low (i.e., <2 mg/L), while those at distances >8 km fluctuated strongly

on a diel basis and rarely fell below 2 mg/L (**Figure 3-14**).

Cluster analyses of mean and minimum daily DO concentrations at these stations showed distinct differences between unenriched (E5, F5, U1, U2, and U3) and enriched stations (E1, E2, E3, E4, F1, F2, F3, and F4) (**Figure 3-15**), and the decline in both of these parameters at enriched stations was statistically significant across all dates ($p < 0.001$, repeated-measures ANOVA). Change-point analysis also indicated a significant change in these parameters between approximately 8 and 10 km downstream, where mean DO declined from average background concentrations of >4 mg/L to <2 mg/L and minimum DO declined from >3 mg/L to <1 mg/L.

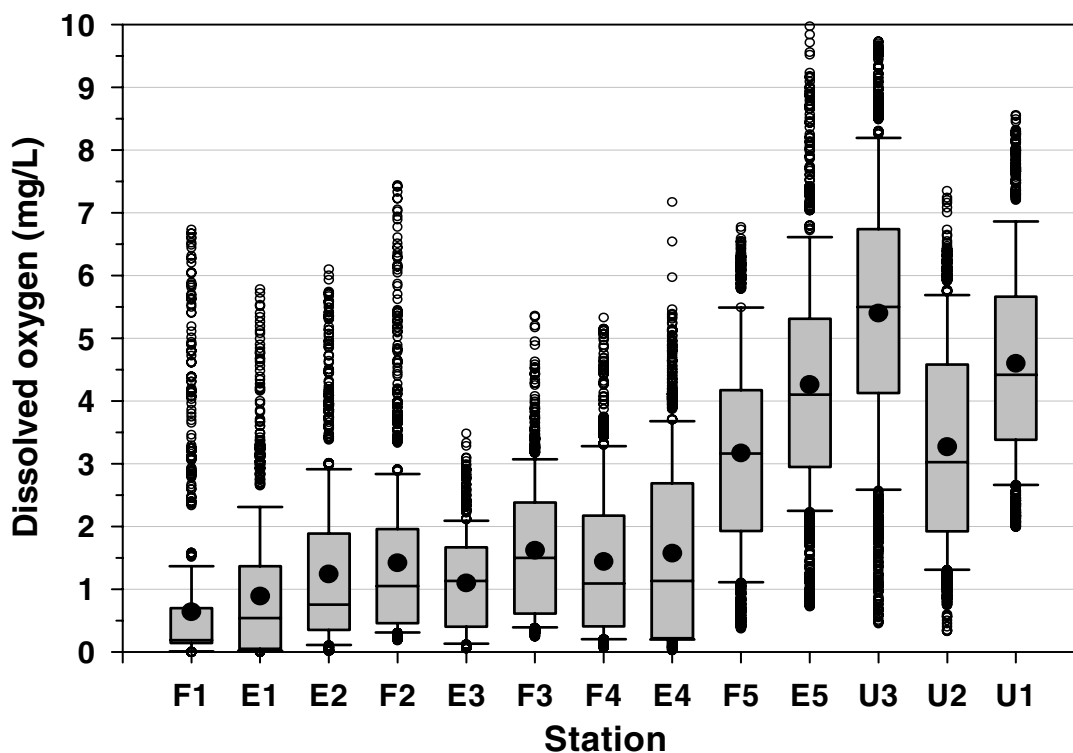


Figure 3-14. Box plots of water column DO concentrations at District transect stations in WCA-2A collected every 15-30 minutes for three to four day periods of time during five sampling periods between April 1995 and February 1998. See **Figure 3-4** for interpretation of box plots.

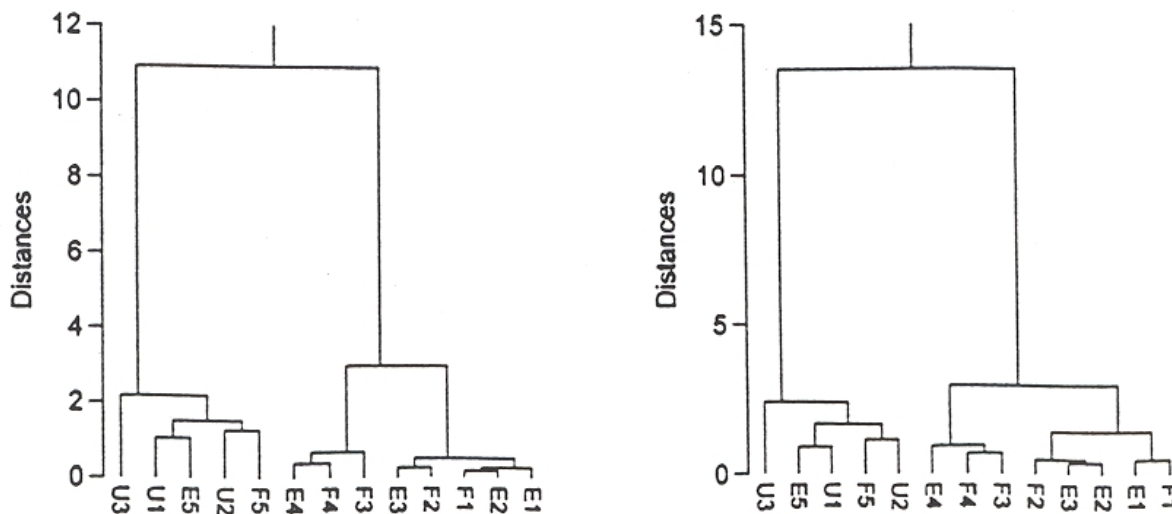


Figure 3-15. Results of cluster analysis (Ward's method) of daily mean and minimum water column DO concentrations at District transect stations in WCA-2A during five sampling periods between April 1995 and February 1998.

Results from a controlled field dosing study conducted by the District provided further evidence of a causal relationship between P enrichment and DO. Diel oxygen measurements were recorded on 16 occasions between August 1995 and April 1998 in mesocosms dosed with different P loads (McCormick and Laing, in review). Diel oxygen fluctuations were similar across all treat-

ments during the first year of dosing, but declined significantly below unenriched levels at P loads of $1.6 \text{ g P/m}^2/\text{yr}$ or greater after three years of enrichment (**Figure 3-16**). These declines were attributed to reduced periphyton/submerged aquatic vegetation photosynthesis, increased SOD, and increased cover of floating and emergent macrophytes as also observed along the marsh gradient.

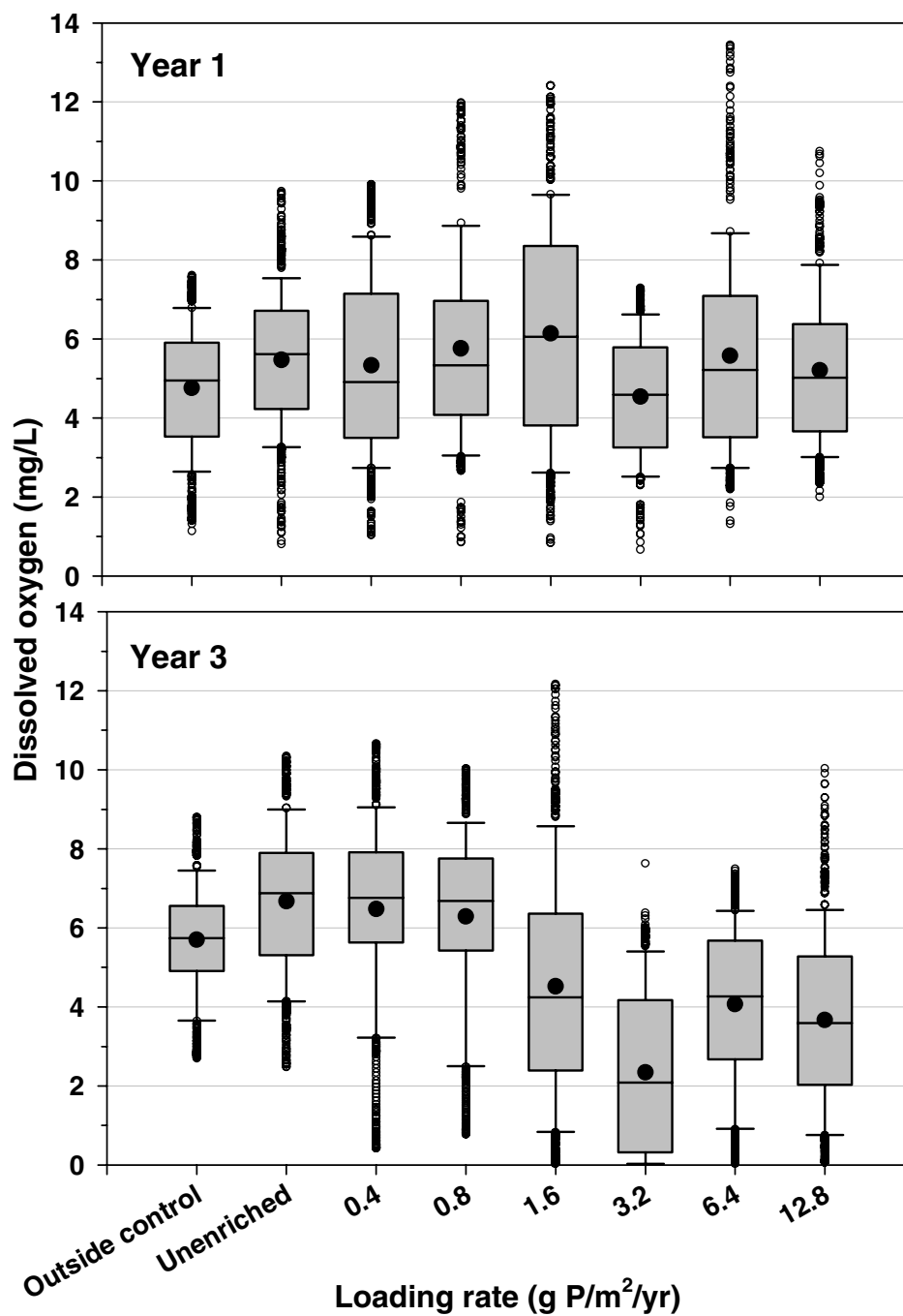


Figure 3-16. Box plots of water column DO concentrations in District mesocosms in WCA-2A collected during the first year and third year of P loading. See **Figure 3-4** for interpretation of box plots.

CONCLUSIONS

Analyses of WCA-2A data are ongoing and, to date, have focused primarily on DEP and District data sets. Future analyses will also consider DUWC data sets once DEP determines that QA/QC problems associated with these data have been resolved. Analyses completed to date show that a number of ecological changes occur along the P gradient in this marsh including shifts in periphyton, macrophyte, and invertebrate taxonomic composition as well as changes in microbial activity and water column DO. Many of these changes occur at stations approximately 7-8 km from the canal where water column and soil TP exceed background concentrations, which average around 10 µg/L and 500 mg/kg, respectively. Some of the most dramatic and abrupt changes along this gradient include:

- Replacement of the oligotrophic periphyton community by a community indicative of nutrient-enriched conditions;

- Alteration of vegetation, including the loss of wet prairie and slough species and the invasion of these habitats by cattail;
- Shifts in macroinvertebrate species composition toward dominance by populations tolerant of low DO conditions; and
- Declines in water column DO, including an increase in the frequency and persistence of periods of abnormally low DO.

The focus of most analyses on periphyton, plant, and invertebrate populations is consistent with the approach for criterion development recommended by US EPA (1992). Effects of P enrichment on higher trophic levels, including larger fish and wading birds, has not been studied extensively. However, available information suggests that enrichment effects on these larger predators may be driven by: (1) changes in prey abundance, which may increase with enrichment; and (2) changes in habitat structure, particularly an increase in the coverage of dense emergent macrophyte stands, which may reduce foraging activity by wading birds in enriched areas.

LOXAHATCHEE NATIONAL WILDLIFE REFUGE: PRELIMINARY RESEARCH FINDINGS

The District initiated gradient and mesocosm studies in the Refuge in 1996. Data are being collected in a manner similar to those in WCA-2A to allow for comparisons between the two marshes. Both the Refuge and WCA-2A are oligotrophic marshes that have received inputs of nutrient-enriched canal waters from the same sources for more than three decades. However, whereas mineral-rich canal discharges contribute a large portion of the water entering WCA-2A, the hydrology of the Refuge interior is driven largely by rainfall, which has a very low mineral content. Thus, interior waters of WCA-2A are rich in major ions such as calcium, carbonate, and sodium (i.e., hard-water) whereas those of the Refuge are extremely low in these ions (i.e., soft-water). These differences in background water chemistry (**Table 3-3**) affect the types of biota found in the two marshes and also may affect the sensitivity of the two systems to P enrichment. Therefore, a major focus of data collection and analyses described below has been to compare responses to P enrichment between the Refuge and WCA-2A.

TRANSECT SAMPLING

Eleven sampling stations (two canal stations and nine marsh stations) were established along a nutrient gradient in the southwestern corner of the Refuge (**Figure 3-17**). Canal waters in this area originate as discharges from the S6 and the S5A pump stations. Biological and chemical sampling at these locations commenced in April 1996 and currently are planned to continue through 2000.

Surface Water Chemistry

Surface-water samples were collected monthly by helicopter. Marsh stations were sampled only when water depths exceeded 10 cm. Data presented here were collected between April 1996 and December 1998. As is true across much of the

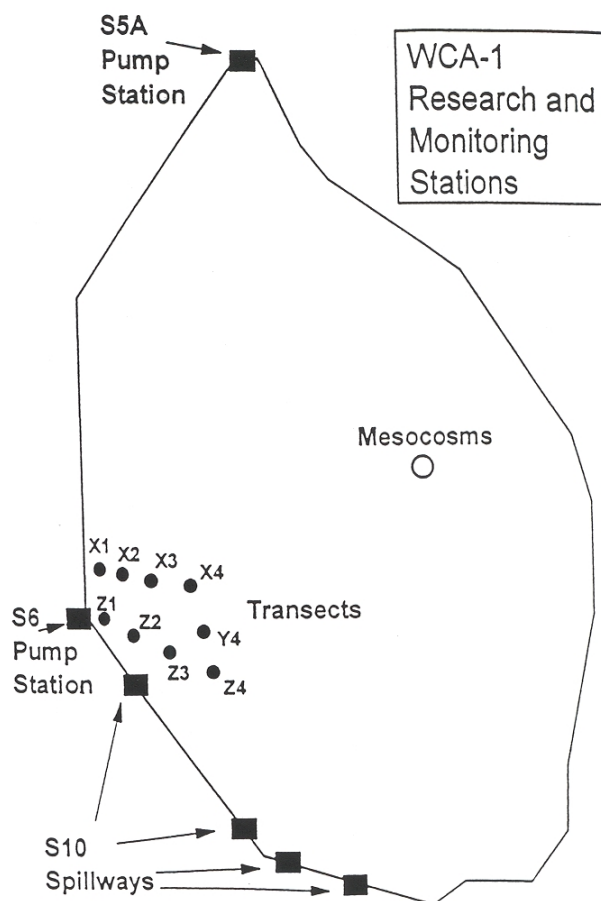


Figure 3-17. Location of District transect stations and mesocosms in Refuge.

Everglades region (Bechtel et al., 1999; McCormick et al., 1999), canal waters entering the marsh are elevated in P and many other elements. Water column P concentrations declined approximately six-fold within the first 2-3 km marsh away from the canal (**Figure 3-18**). As previously shown in WCA-2A (McCormick et al., 1996), the extent of these declines was much greater than for other elements. Mean TP concentrations during the period

Table 3-3. Water column chemistry at interior District transect stations in the Refuge (X4, Y4, and Z4) and WCA-2A (E5, F5, U1, U2, and U3). Numbers are mean values for data collected at stations in each area between 1996 and 1998. See **Figures 3-2** and **3-17** for station locations.

Parameter	Units	Refuge	WCA-2A
Alkalinity	mg/L	44.2	231
Specific conductivity	μS/cm	184	883
pH	pH units	6.45	7.39
Total P	μg/L	11	10
Total dissolved P	μg/L	5	5
Soluble reactive P	μg/L	4	5
Total N	mg/L	1.0	2.1
Total dissolved N	mg/L	0.96	2.1
Ammonia (NH ₃)	μg/L	28	44
Nitrate (NO ₃)	μg/L	<4	9
Nitrite (NO ₂)	μg/L	<4	<4
Calcium	mg/L	13.0	68.7
Magnesium	mg/L	4.40	24.6
Potassium	mg/L	1.33	6.47
Sodium	mg/L	18.8	89.9
Iron	μg/L	20	9.0
Sulfate (SO ₄)	mg/L	2.8	45
Silica (SiO ₂)	mg/L	6.8	16
Chloride	mg/L	28	127
Dissolved organic carbon	mg/L	18	36
Total suspended solids	mg/L	<4	<4

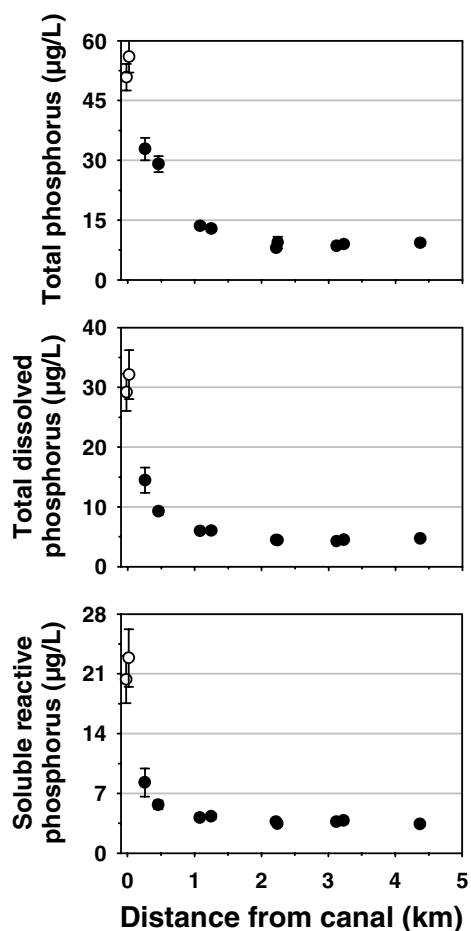


Figure 3-18. Mean (± 1 SE) water column phosphorus concentrations in samples collected between April 1996 and September 1998 at Refuge transect stations with increasing distance from perimeter canal.

of record ranged between 51 and 56 $\mu\text{g/L}$ at canal stations and declined to between 8 and 9 $\mu\text{g/L}$ at marsh stations >2 km from the canal. Total dissolved P (TDP) and soluble reactive P (SRP) averaged 29-32 and 20-23 $\mu\text{g/L}$, respectively, in the canal and declined to 4-5 and 3-4 $\mu\text{g/L}$, respectively, in the marsh interior.

Concentrations of total nitrogen (TN), a nutrient which can sometimes limit algal growth in fresh waters, averaged 2.3-2.6 mg/L in the canal

and declined to 1.0-1.3 mg/L at stations >2 km into the marsh (**Figure 3-19**). Nitrogen concentrations in the Refuge were somewhat lower than those in WCA-2A but were well above levels that might indicate N-limitation. The TN:TP ratio provides an approximate indication of the nature of limitation, with low values (e.g., <8:1 w:w) suggesting N limitation and higher values suggesting P limitation. The ratio of TN:TP increased from near 50:1 in the canal to near 150:1 in the marsh interior. These data suggest that: (1) P is the more important limiting nutrient in both the marsh and canal waters; and (2) severity of P limitation increases with increasing distance from the canal. These trends are similar to those found along nutrient gradients in WCA-2A (McCormick et al., 1996), and predictions of P limitation are supported by the results of algal bioassays (see below).

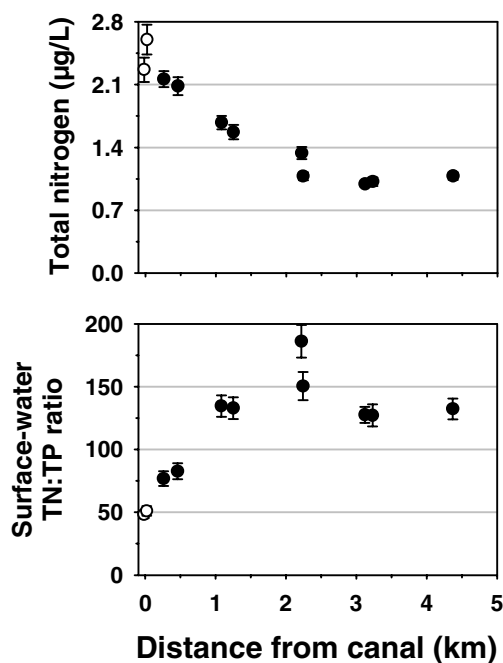


Figure 3-19. Mean (± 1 SE) water column total nitrogen concentrations and N:P ratios in samples collected between April 1996 and September 1998 at Refuge transect stations with increasing distance from the perimeter canal.

Canal waters had a higher ionic content than the marsh as illustrated by changes in conductivity, alkalinity, and the concentration of major cations such as calcium and sodium along the gradient (**Figure 3-20**). This pattern reflects the different origins of water in the canal (agricultural runoff, groundwater) and the marsh interior (rainfall). Concentrations of these major ions were strongly correlated with each other and declined by roughly 80 percent between the canal and the most interior marsh stations (>3 km from the canal). Whereas declines in P and N along the gradient were rather steep as a result of active biological removal and transformation, concentrations of major ions declined more slowly away from the canal. This pattern suggests that dilution, rather than active biological removal, is the principal cause of these declines in parameters other than N and P.

Overall, water quality patterns along enrichment gradients in the Refuge are similar to those in WCA-2A in that waters near the marsh periphery contain higher concentrations of P and other nutrients and are more mineralized than those in the marsh interior. However, the water quality gradient in the Refuge is much steeper, indicating that canal waters do not intrude as far into the marsh as in WCA-2A. As documented in WCA-2A, P concentrations decline much more rapidly than other elements with distance from the canal, likely due to chemical sorption and biological uptake of this limiting nutrient. Declines in elements other than P along the water-quality gradient in the Refuge are much steeper than in WCA-2A. This difference is attributable to the rainfall-driven hydrology of the Refuge interior, which contributes to extremely low concentrations of most ions in the water column. By contrast, canal discharges account for much of the water entering WCA-2A, thus explaining the mineralized condition of that marsh.

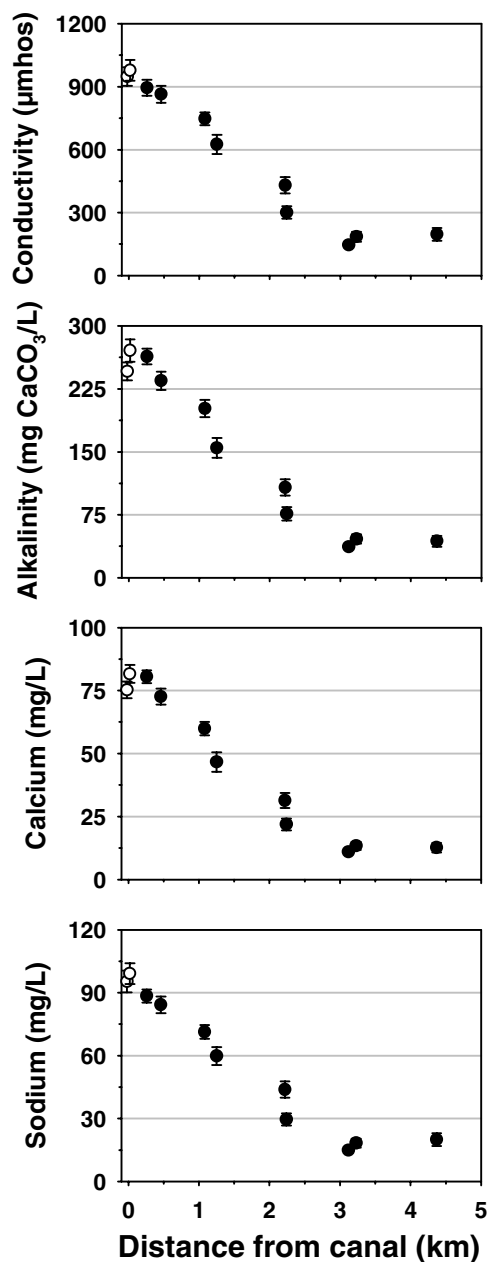


Figure 3-20. Mean (± 1 SE) water column conductivity and concentrations of major ions in samples collected between April 1996 and September 1998 at Refuge transect stations with increasing distance from perimeter canal.

Soils and Porewater

The soil nutrient gradient in the Refuge is not as extensive as observed in WCA-2A, with signs of soil P enrichment confined to stations within 2 km from the canal (**Figure 3-21**). In general, soil TP

concentrations decrease with soil depth, with no apparent effects of P enrichment in the 10-30 cm depth increment. TP concentrations at interior stations are <500 mg/kg, which are similar to those measured at unenriched stations in WCA-2A and WCA 3 (DeBusk et al., 1994; Reddy et al., 1994).

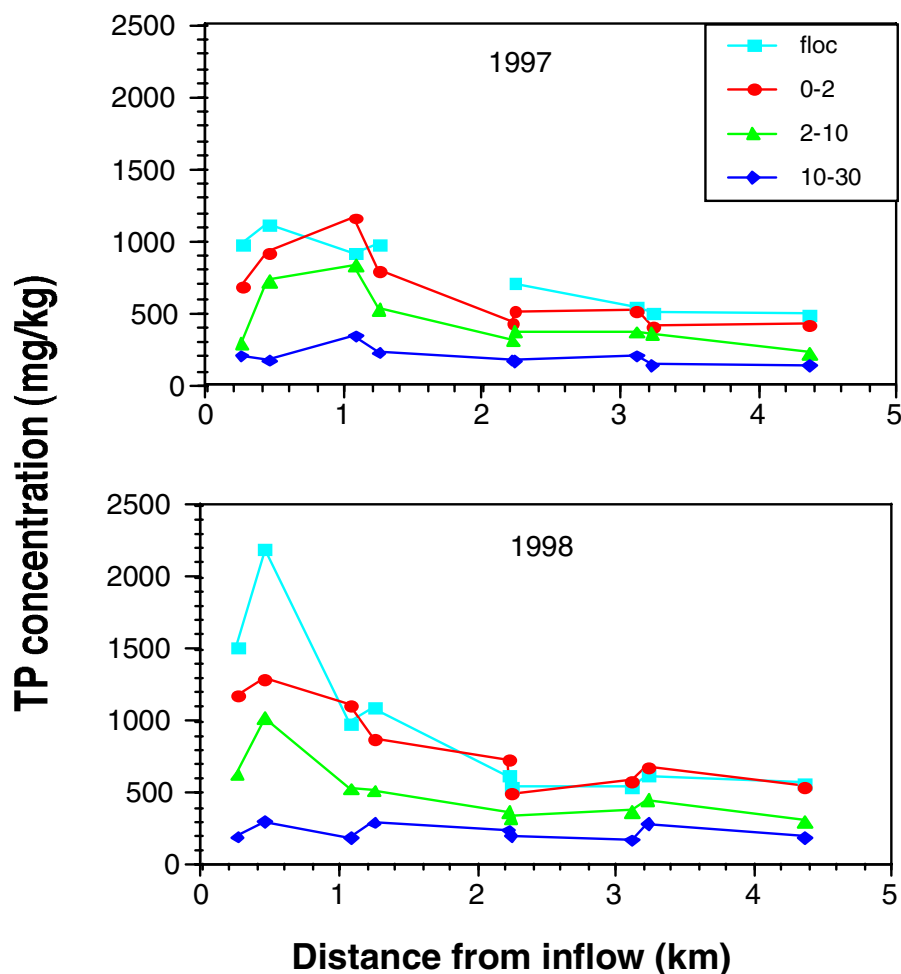


Figure 3-21. Vertical profiles of soil TP in samples collected during February 1997 and April 1998 at Refuge transect stations with increasing distance from perimeter canal.

While water column and soil TP gradients in the Refuge are steeper than in WCA-2A, there are comparable relationships between soil and water column TP concentrations. Soil TP concentrations increase rapidly at water column TP concentrations ranging 10-20 $\mu\text{g/L}$ and level off at concentrations >40 $\mu\text{g/L}$ (**Figure 3-22**). Phosphorus accumulation in the soil profile consolidates decades of nutrient enrichment, thus a direct comparison with present day water chemistry to determine a threshold value is not appropriate. However, the data do suggest that moderate water column TP enrichment may ultimately cause increased soil P enrichment.

Concomitant with soil P, porewater TDP and TDKN show distinct concentration decreases with increasing distance from the canal (**Figure 3-23**). Maximum P and N concentrations occur within 1 km of the canal and decrease to concentrations of 0.004 to 0.01 and 0.58 to 1.8 mg/L, respectively, at interior marsh stations. SRP concentrations follow the same trend as TDP, and greater than 90 percent of TDP is present as SRP, suggesting that the majority of TDP may be bioavailable. While $\text{NH}_4\text{-N}$ concentrations also closely follow the TDKN trend, less than 50 percent of TKN is present as $\text{NH}_4\text{-N}$.

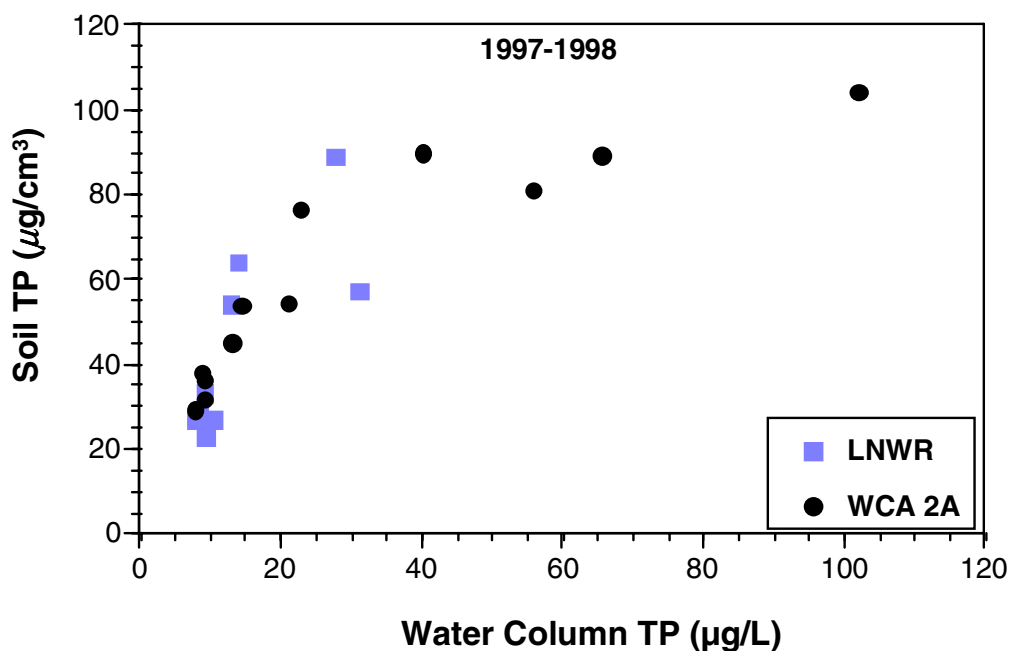


Figure 3-22. The relationship between soil TP and water column TP along nutrient gradients in the Refuge and WCA-2A. Soils data are the mean of 2 sampling events. Water column data represent means of biweekly and monthly sampling between January 1997 and December 1998.

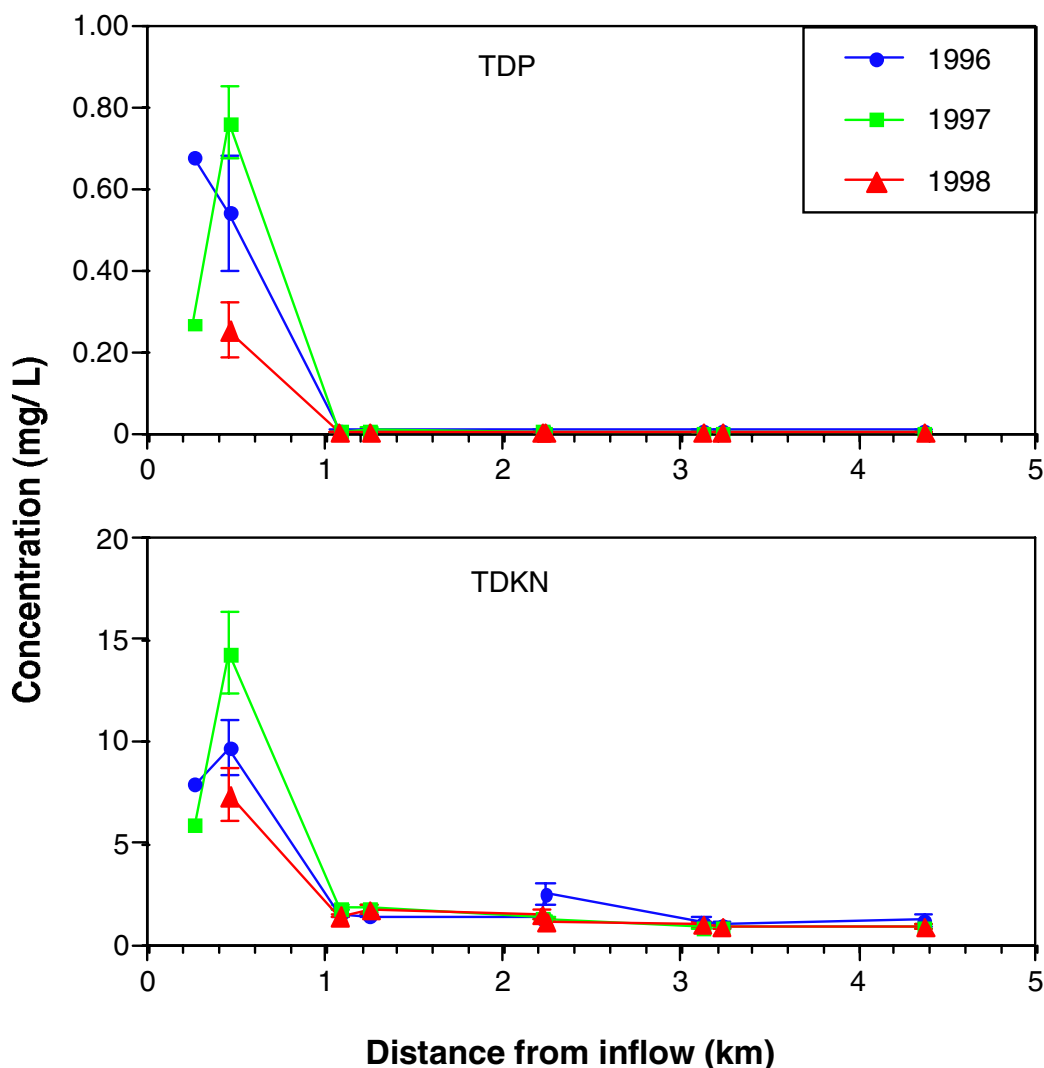


Figure 3-23. Total dissolved P and TDKN concentrations (mean ± 1 SE) in porewater samples collected quarterly between June 1996 and September 1998 in wells (2-12 cm) at Refuge transect stations with increasing distance from perimeter canal.

With the exception of Fe, an exponential decline in other nutrients and metals is observed with increasing distance from inflow, resulting in an order of magnitude decrease (**Figure 3-24**). In general, the greatest changes in porewater nutrient concentrations occur within the first 3 km and then level off. Unlike porewater N and P concentrations, the concentrations of major cations at WCA-2A and Refuge marsh stations are considerably differ-

ent. With the exception of Refuge stations within 1 km of the canal, porewater calcium and magnesium concentrations in the Refuge average <50 and <10 mg/L, respectively; two- to five-fold lower than those observed in WCA-2A. In association with nutrient chemistry, porewater pH also decreases with distance from the canal, decreasing 0.5 units from an average of 6.7 at the canal stations to 6.2 at the interior stations.

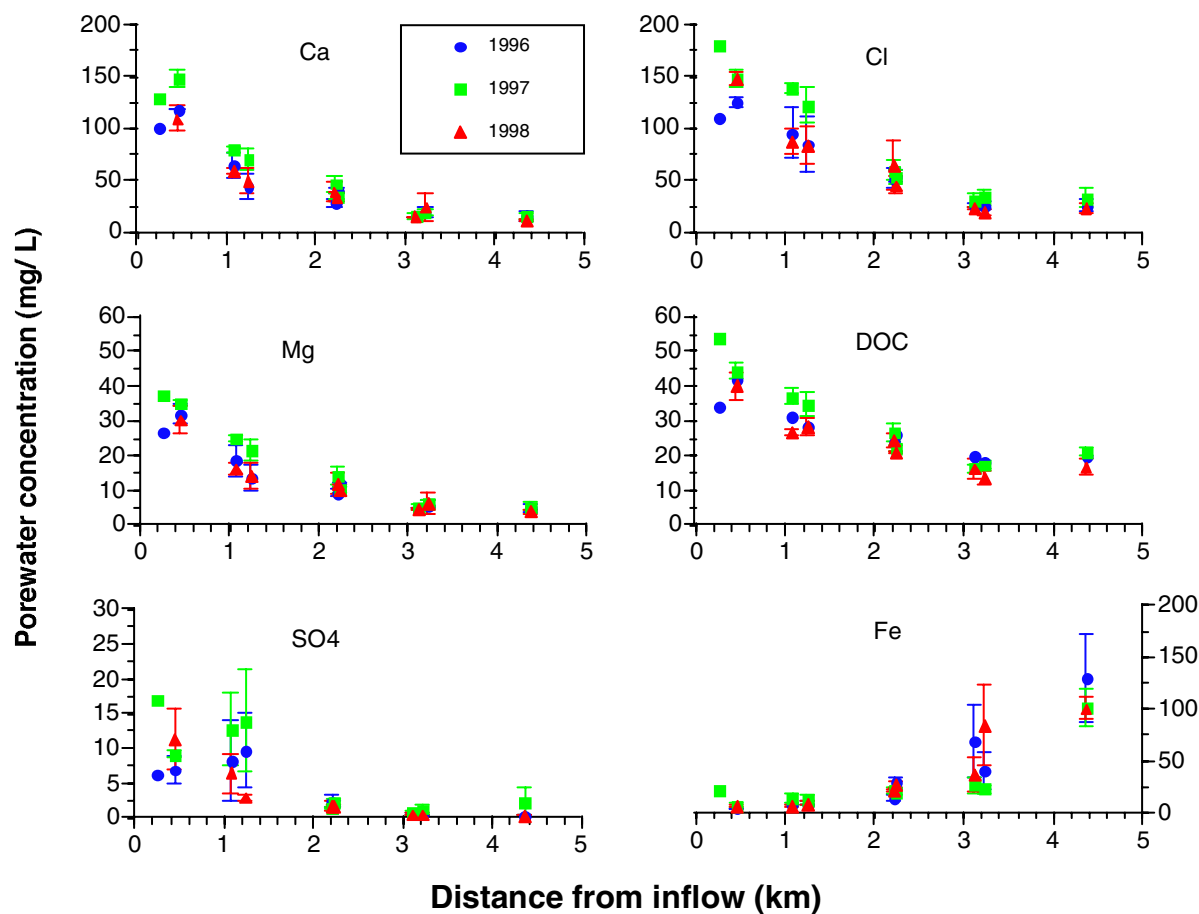


Figure 3-24. Concentrations of Ca, Cl, Mg, DOC, SO₄, and Fe (mean ± 1 SE) in porewater samples collected quarterly between June 1996 and September 1998 in wells (2-12 cm) at Refuge transect stations with increasing distance from perimeter canal.

In contrast to other parameters measured, both Fe and redox values increase with increasing distance from the canal (**Figures 3-24 and 3-25**). Fe concentrations increase exponentially from less than 50 $\mu\text{g/L}$ at stations close to the canal to >200 $\mu\text{g/L}$ at stations within the marsh interior. Lower concentrations near the canal may be due to micro-

bial uptake caused by increased metabolism, although this has not been ascertained. Redox values vary seasonally, but are more reduced at canal stations. Redox values average -67 mv at 0.46 km from the canal and increase to 121 mv at 4.37 km away from the canal.

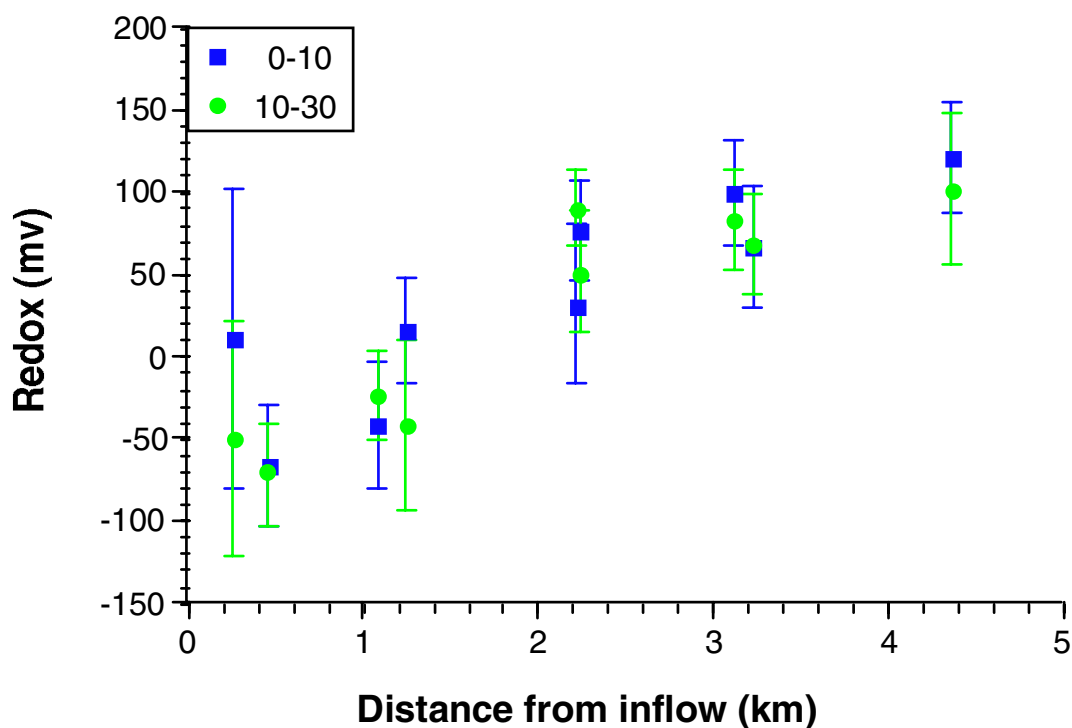


Figure 3-25. Redox values (mean ± 1 SE) collected quarterly between June 1996 and September 1998 in surface soils at Refuge transect stations with increasing distance from perimeter canal.

Diel Oxygen Profiles

Hydrolabs™ were deployed periodically at all marsh stations to measure diel fluctuations in water column DO. Hydrolabs™ were suspended from tripods situated at the edge of open-water habitats with the oxygen probe situated at approximately mid-depth in the water column. Deployments generally occurred on Monday before noon and retrievals generally were made on Friday afternoon to capture four complete diel cycles. Measurements of DO were collected every 30 minutes during this period. Measurements presented here were performed during 1997 and 1998 and included two wet season (June 1997, October 1997) and one dry season (February 1998) sampling periods.

As in WCA-2A, unenriched areas of the marsh interior typically maintain higher DO concentrations than enriched locations near canal inputs (**Figure 3-26**). Mean DO during the three sampling periods ranged between 2 and 8 mg/L at interior stations as compared with concentrations <2 mg/L at stations closest to the canal. Minimum DO generally was >1 mg/L at interior stations, but approached anoxia (0 mg/L) at stations closest to the canal. The frequency of extremely low DO (<1 mg/L), which might be stressful to native plant and animal populations, generally was low (<25 percent of readings) at interior stations, but was quite high (60-100 percent of readings) at stations near the canal.

Patterns of DO decline with P enrichment were similar in the Refuge and in WCA-2A (**Figure 3-27**). Rates of decline in mean and minimum DO and increase in the frequency of DO <1 mg/L were greatest in areas of both marshes where water column TP increased from approximately 10 to 20 µg/L suggesting a transition from the reference to the enriched condition within this TP range. The causes and consequences of changes in water column DO concentrations are discussed in greater detail by McCormick and Laing (in review). The dynamics of DO relative to water quality standards are discussed in **Chapter 4**.

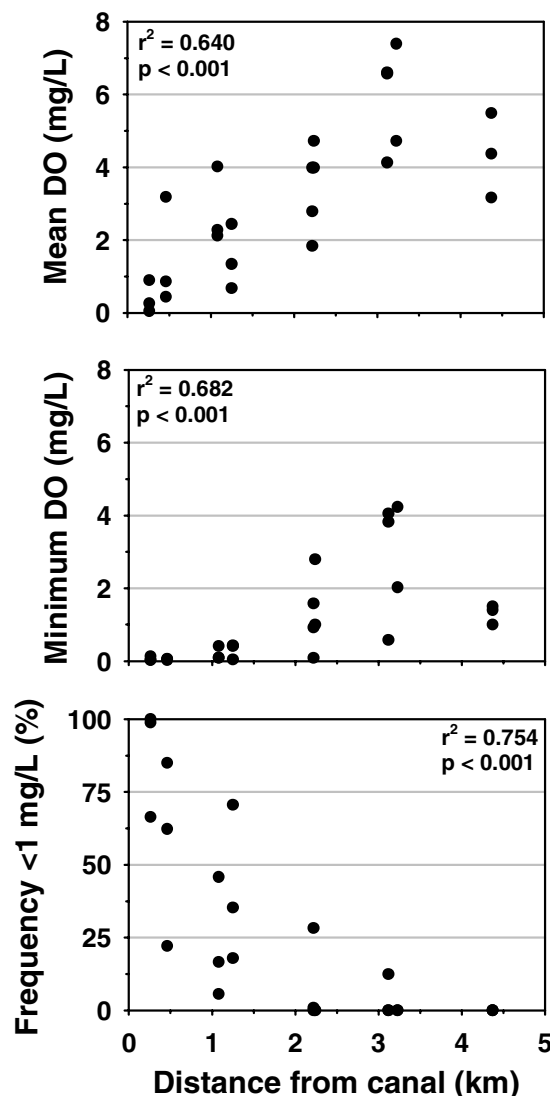


Figure 3-26. Mean and minimum water column DO concentrations and frequency of abnormally low (<1 mg/L) concentrations at Refuge transect stations with increasing distance from perimeter canal. Values are based on measurements collected over multiple diel cycles during three separate sampling periods between June 1997 and February 1998.

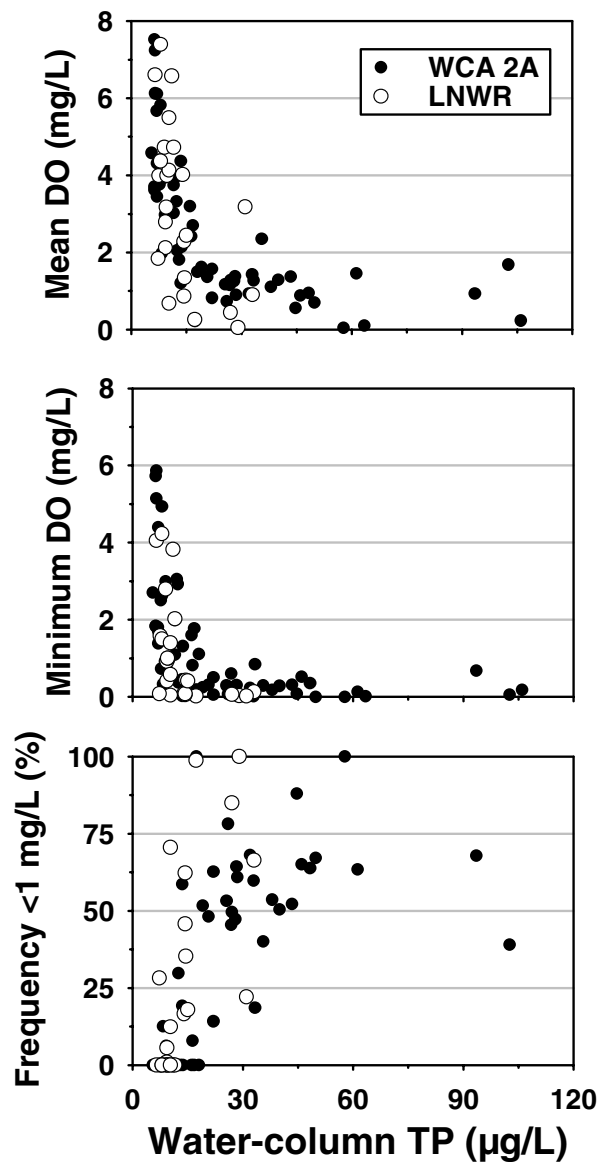


Figure 3-27. Comparison of changes in mean and minimum water column DO concentrations and the frequency of abnormally low (<1 mg/L) concentrations as a function of water column TP concentration along nutrient gradients in WCA-2A and the Refuge. Values are based on measurements collected over multiple diel cycles during five separate sampling periods between April 1995 and February 1998 in WCA-2A and three separate sampling periods between June 1997 and February 1998 in the Refuge.

Microbial Processes

Phosphatase Activity. Phosphatase activity (PA), specific enzymatic activity generated in response to P limitation, has been recommended as a rapid and simple means to assess the P status of aquatic systems (Healey & Hendzel, 1980; Whittton, 1991). A previous study showed that water column PA varied across regions and tended to be higher in the Refuge and ENP, which may suggest these areas are extremely P limited (US EPA 1998). A comparison of PA collected as part of the District's water quality monitoring network across the Everglades confirmed that interior stations in the Refuge had higher PA than those in the interior of WCA-2A and WCA-3A (**Figure 3-28**). No data were collected from enriched Refuge stations, therefore, a comparison of repression is not possible. However, in WCA-2A, PA was repressed in

response to P enrichment, decreasing to at or below the 5 nmol/min/mL detection level at water column TP concentrations $>10 \mu\text{g/L}$ (**Figure 3-28**, inset).

Decomposition Rates. Higher PA rates in the water column might suggest higher microbial decomposition rates. However, decomposition rates, as determined using cotton strip tensile strength loss, were similar in both the Refuge and WCA-2A (**Figure 3-29**). In contrast to PA, an indicator of P limitation, decomposition rates are stimulated by P enrichment. At reference stations mean cotton strip tensile strength loss rates above the soil surface ranged 1-4 percent/day, while at enriched stations cotton strip tensile strength loss rates were 4-7 percent/day. A similar increase in cotton strip tensile strength loss rates below the soil surface also was observed in response to soil P enrichment, although the relationship appeared more linear

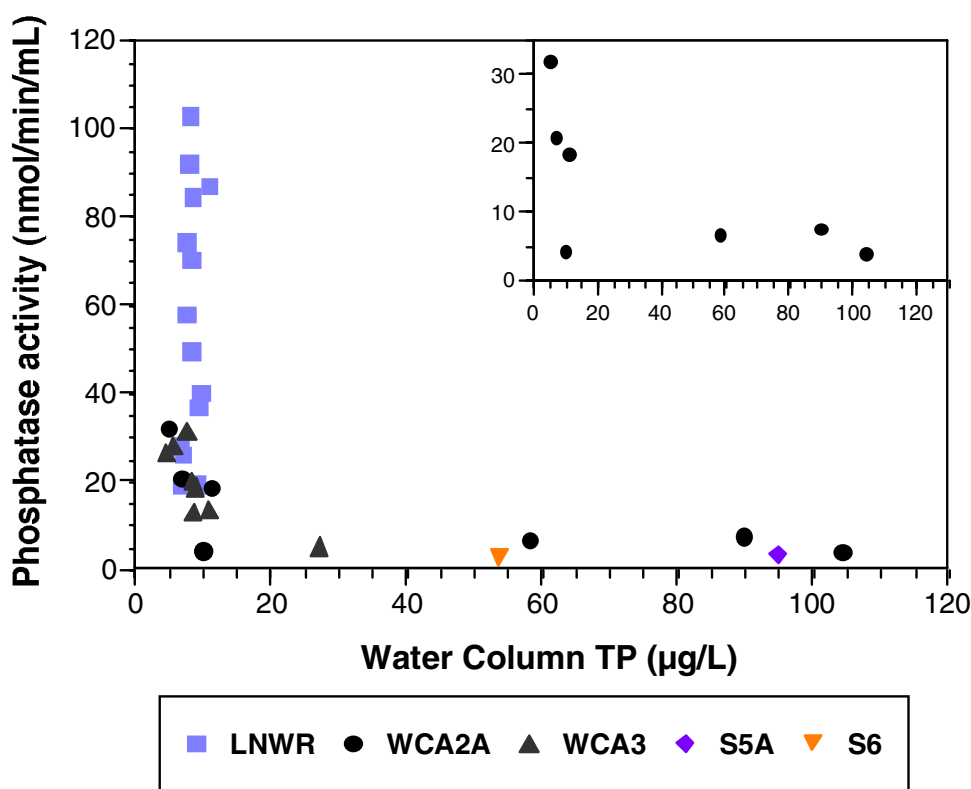
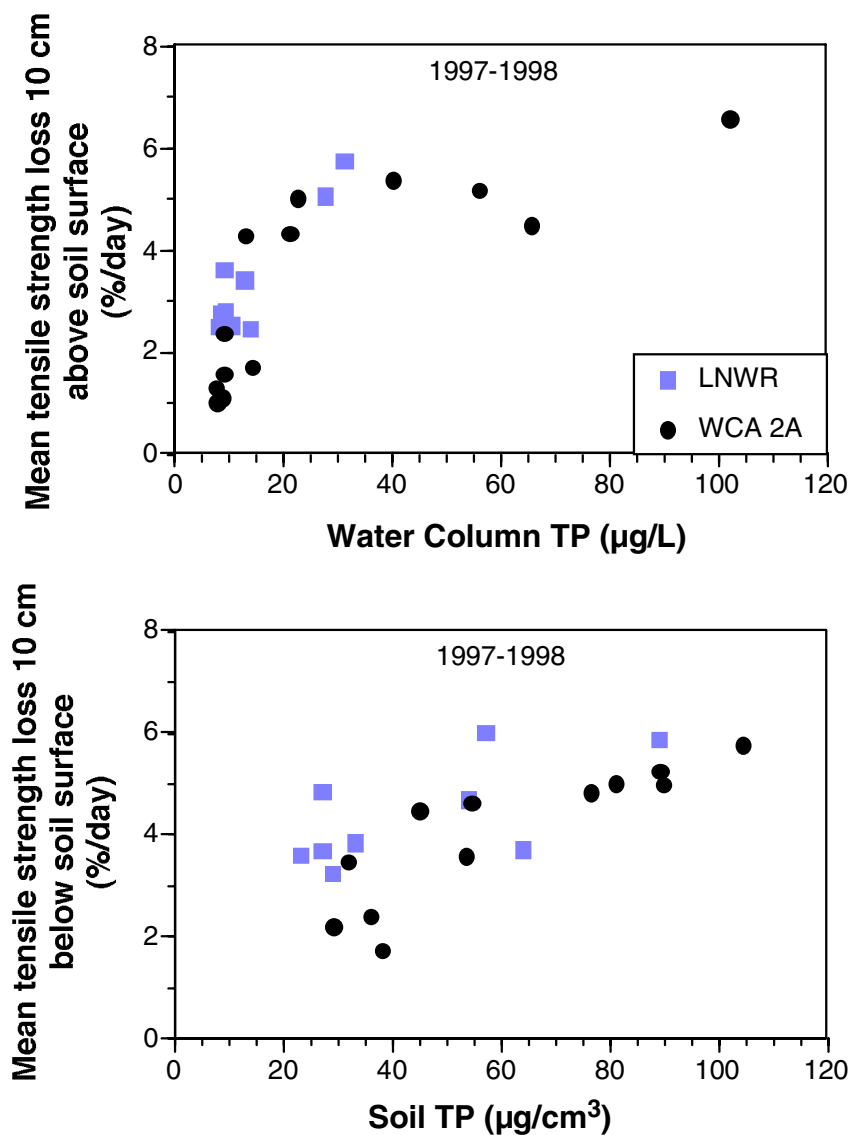


Figure 3-28. Water column phosphatase activity as a function of water column TP in several areas throughout the Everglades. Data are means of samples collected biweekly between February 1995 and December 1998. Inset highlight decrease in PA observed with increased TP in WCA-2A.

(Figure 3-29). Previous work in ENP supports this response to P enrichment. In flumes with P and N+P additions an increase in decomposition was observed, while enrichment with N alone produced no decomposition response within the 12-month study period (Maltby, 1985). This suggests that

once the temporal and spatial variability of decomposition rates at unenriched stations is established, changes in soil decomposition rates may be used to indicate the effects of P load in the soil, prior to any detectable change in soil chemistry.



Algal Growth Potential and Limiting Nutrient Assays

Unfiltered water samples were collected every two months from each marsh and canal station and used in bioassays to determine changes in the nature and extent of nutrient limitation along the Refuge enrichment gradient. Samples were first sterilized by autoclaving and then filtering through a 0.45- μ m membrane filter. Sterilized samples were used to conduct the standard *Selenastrum capricornutum* Printz Algal Assay Bottle Test (US EPA, 1978). *Selenastrum capricornutum* is a chlorophyte, or “green” alga, related to some of the algal taxa found in the Refuge. A known amount of *S. capricornutum* was added to triplicate flasks of sterilized water from each station. Flasks were incubated under controlled laboratory conditions to determine the biomass (mg dry weight/L) of algae produced in each water sample after 14 days. This yield is termed the algal growth potential (AGP) and can be used to infer relative differences in the availability of growth-limiting nutrients in waters collected from different stations. An additional portion of each water sample was sterilized and used to conduct a limiting nutrient algal assay (LNAA). Subsamples were amended with 1000 μ g/L N, 100 μ g/L P, and 100 μ g/L EDTA (to enhance micronutrient availability), both alone and in combination. The yield of *S. capricornutum* was compared among nutrient treatments for each water sample to determine the nutrient(s) most limiting to algal growth (i.e., dry weight production).

Limiting nutrient assays indicated that P was the principal nutrient limiting algal growth throughout the marsh, with the exception of one occurrence of N and P co-limitation at the station closest to the canal (Z1) (**Figure 3-30**). Phosphorus also was the principal limiting nutrient in the canal on most occasions; however, a switch to N limitation occurred on certain sampling dates and was associated with periods when P concentrations were highest. Overall, these findings for the Refuge are consistent with those presented for WCA-2A by McCormick et al., 1996, who found a shift away from P limitation only at marsh stations

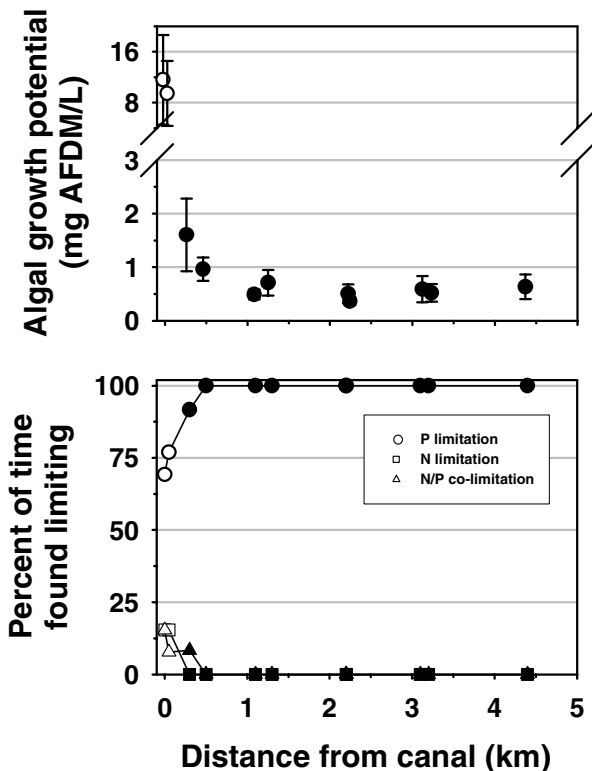


Figure 3-30. Results of standardized algal bioassays conducted using surface water collected from Refuge transect stations to assess changes in the algal growth potential (AGP) and the nature of nutrient limitation with increasing distance from the perimeter canal. Open symbols show data from canal stations. See text for a description of these methods.

where P concentrations exceeded those commonly measured along the Refuge gradient.

Results from samples collected between April 1996 and July 1998 showed substantial changes in AGP along the gradient. Algal growth potential was similar among stations located >1 km from the canal and averaged between 0.36 and 0.71 mg dry weight/L (**Figure 3-30**). Marsh stations closest to the canal (X1 and Z1) had average AGP values that were two- to three-fold higher than those at more

interior stations. These elevated levels are similar to those measured at moderately enriched marsh stations in WCA-2A, but are considerably lower than those measured at the most enriched stations in WCA-2A (McCormick et al., 1996), which have water column P concentrations that exceed those found along the Refuge gradient. Canal waters, which contained extremely high concentrations of P and other nutrients, had an AGP that was highly variable but, on average, more than 20-fold higher than that of waters in the marsh interior.

As predicted by LNAA results, a strong relationship existed between AGP and water column TP concentrations in both the Refuge and WCA-2A (**Figure 3-31A and B**). Over a range of TP concentrations between 5 and 100 $\mu\text{g/L}$, AGP increased roughly 100-fold. Linear regression analysis was used to compare AGP TP relationships in the two marshes and, as shown in **Figure 3-31C**, the relationships were nearly coincident. These results indicate that P enrichment has a similar stimulatory effect on algal growth rates despite differences in background water chemistry in the two marshes. Because *Selenastrum* is not a component of Everglades periphyton assemblages, LNAA and AGP results should be viewed only as suggestive of responses of ambient algal taxa.

Periphyton Biomass

Periphytometers were deployed quarterly at each marsh station, retrieved after eight weeks of immersion, and sampled to measure accrued biomass (as chlorophyll *a*) and taxonomic composition. Results presented here are based on data from eight sets of periphytometers collected between June 1996 and June 1998.

Rates of periphyton biomass accrual generally increased with nutrient enrichment and, thus, were consistent with AGP results described above. Chlorophyll (chl) *a* biomass on periphytometers was seasonal, with highest biomass generally occurring during the wet season when temperature, light and nutrient inputs from rainfall were relatively high. Biomass declined sharply with increas-

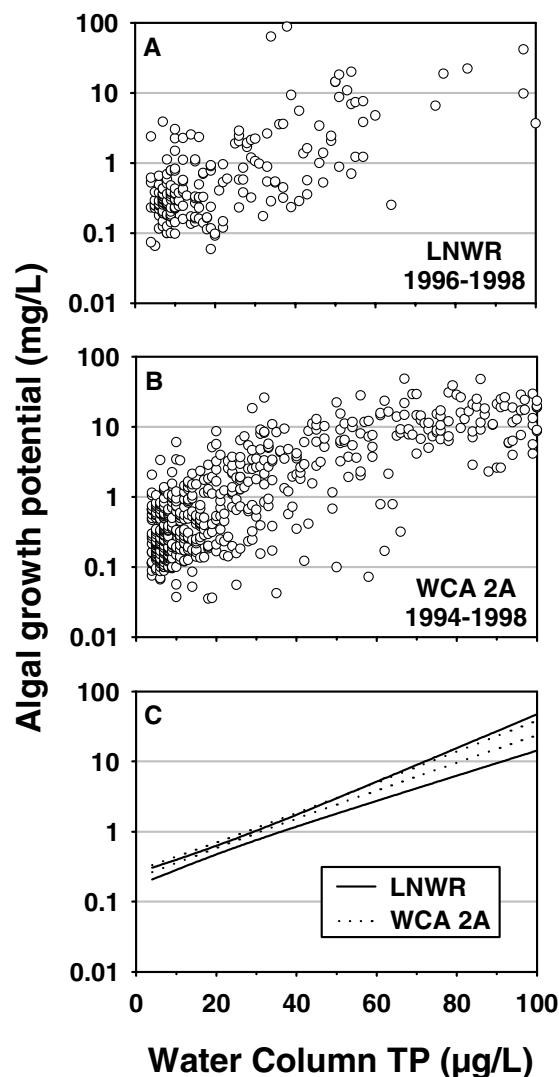


Figure 3-31. Comparison of changes in AGP as function of water column TP concentration along nutrient gradients in the Refuge (**A**) and in WCA-2A (**B**) and 95% confidence intervals (**C**) for linear regressions between water column TP and AGP (log-transformed) for the two marshes. This analysis excluded WCA-2A samples where TP >100 $\mu\text{g/L}$ as concentrations above these levels were never measured in the Refuge. See text for further details.

ing distance from the canal (**Figure 3-32**). Biomass accrual on periphytometers incubated at enriched stations closest to the canal (<1 km) varied among sampling dates, but generally ranged between 5 and 25 mg chl *a*/m². In contrast, stations approximately 1 km from the canal, which had slightly elevated nutrient concentrations, typically accumulated <5 mg chl *a*/m², and nutrient-poor stations in the marsh interior typically accumulated <1 mg chl *a*/m².

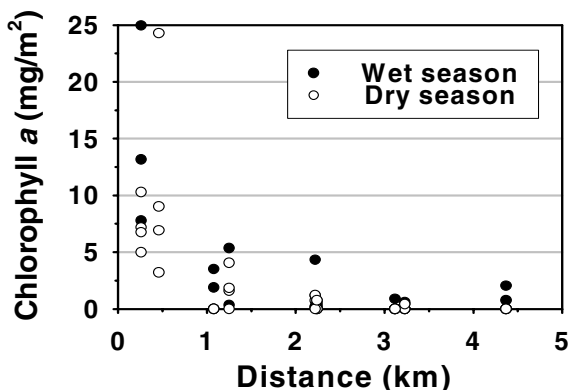


Figure 3-32. Periphyton chlorophyll *a* concentrations on periphytometers collected between June 1996 and June 1998 at Refuge transect stations with increasing distance from perimeter canal.

The relationship between periphytometer chlorophyll *a* biomass and water column TP was similar along enrichment gradients in the Refuge and WCA-2A (**Figure 3-33**). Variability in this parameter in low P areas of both marshes was high, due in part to seasonal fluctuations in growth rates, and typically ranged from near detection to roughly 10 mg/m². The relationship between biomass and increasing enrichment was strongest at water column TP <20 µg/L. At higher TP concentrations, biomass ranged between 1 and 100 mg chl *a*/m² and appeared unrelated to P concentrations.

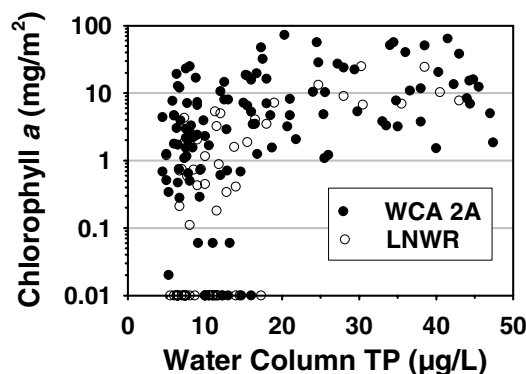


Figure 3-33. Comparison of changes in periphyton chlorophyll *a* as function of water column TP concentration along nutrient gradients in WCA-2A and the Refuge. WCA-2A data, where TP >50 µg/L, were excluded as these levels were never measured in Refuge in association with periphyton sampling.

Periphyton Taxonomic Composition

Preliminary analyses of periphyton taxonomic changes along enrichment gradients in the Refuge have been performed based on natural unit densities of each taxon. One unit is defined as one organism whether that organism is unicellular or a multicellular natural colony or filament (Clesceri et al., 1998). Periphyton natural unit densities on periphytometers decreased with increasing distance from the canal (**Figure 3-34**). As for AGP and biomass, this pattern indicated increasing algal growth rates associated with canal inflows. The three most interior stations (>3 km from the canal) generally had densities <60,000 units/cm². Densities were more variable at stations approximately 1-2 km from the canal and ranged between 30,000 and 120,000 units/cm². Stations closest to the canal had densities ranging between 60,000 and 250,000 units/cm². No clear seasonal pattern was evident as some stations had greater densities during the wet season while others showed dry season maxima.

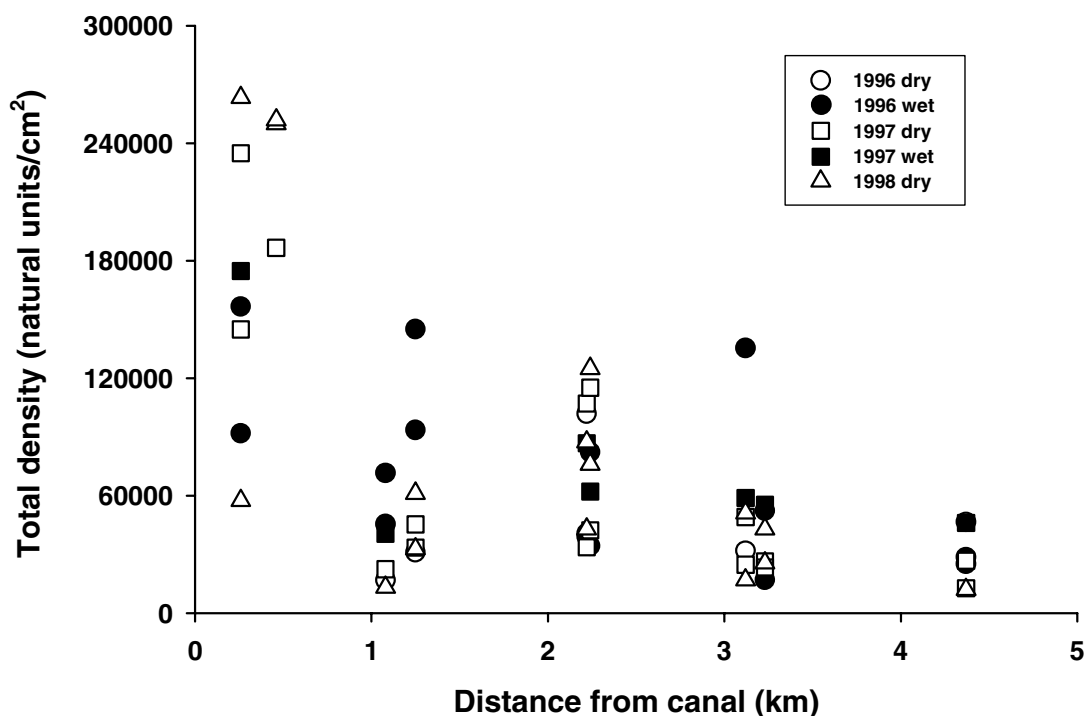


Figure 3-34. Total periphyton density (natural units) on periphytometers collected at Refuge transect stations with increasing distance from the perimeter canal.

The relative abundance of major taxonomic groups varied among sampling dates at all stations, but certain seasonal and spatial patterns were evident (**Figure 3-35**). Cyanobacteria abundance was greatest during the wet season, particularly at hard-water, nutrient-enriched stations near the canal where this group comprised as much as 80-100 percent of the periphyton assemblage during the wet season. By contrast, diatoms and chlorophytes were relatively more abundant during the cooler dry season months. Diatom relative abundance was greatest at hard-water, low-nutrient stations located at intermediate distances from the canal and were lowest at stations near the canal during the wet season. Chlorophyte relative abundance generally was greatest at the most interior stations (soft-water, low-nutrient) and also were low at stations near the canal during the wet season.

One group of chlorophytes, the desmids, is particularly well-represented in the Refuge. This

group of algae is commonly found in waters that are low both in nutrients, such as P, and in other major ions, such as sodium and calcium (Brook, 1981). Along the transects, the relative abundance of this group was greatest at the softwater, low-nutrient stations in the marsh interior, where they comprised as much as 25 percent of the periphyton assemblage (**Figure 3-35**). The relative abundance of this group generally was lower at intermediate stations, where the surface water was low in P, but elevated in other ions, such as calcium and sodium. This group was least abundant at stations <2 km from the canal where concentrations of P and other major ions were elevated relative to the marsh interior. Given the sensitivity of most desmids to increases in ionic content per se, it is not clear to what extent the decline in the abundance of this group along the Refuge gradient is a consequence of P enrichment, as opposed to increases in other ions.

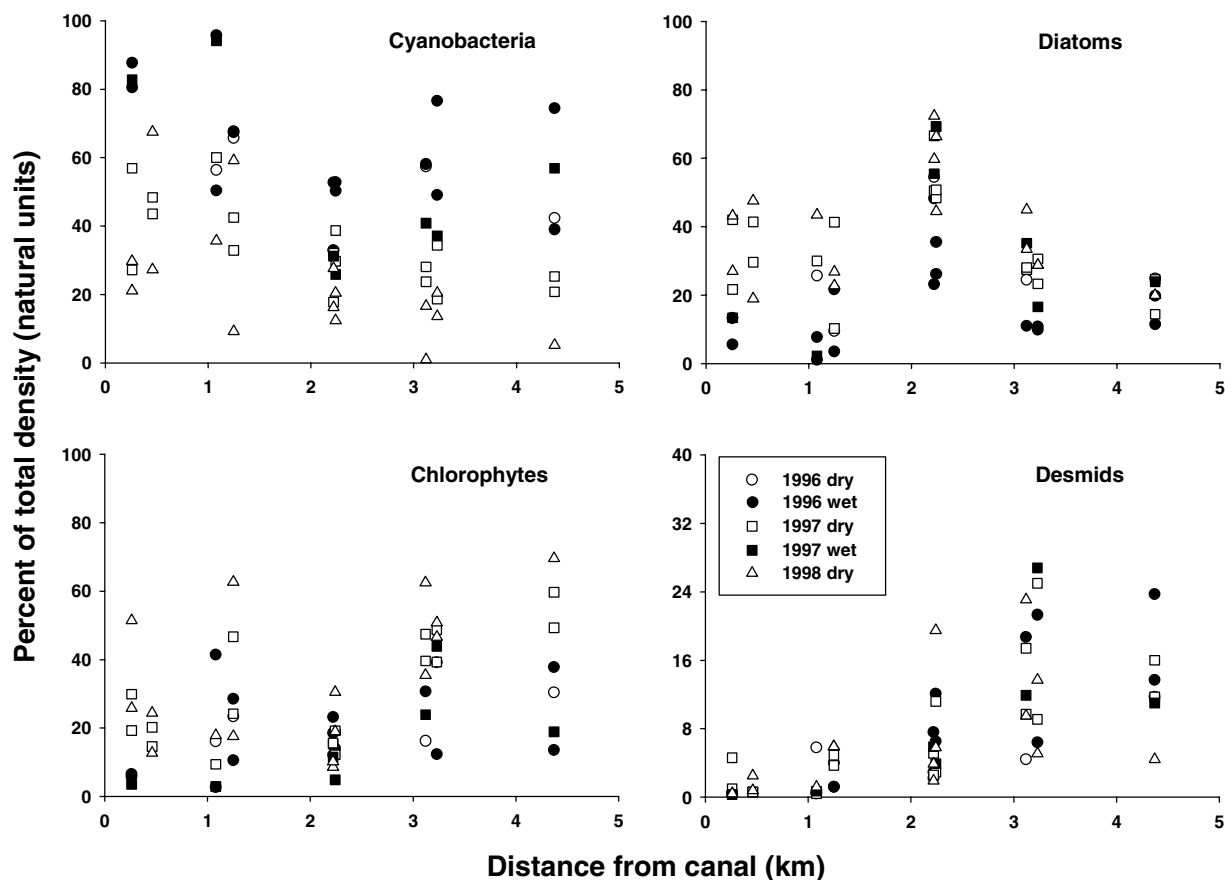


Figure 3-35. Proportional abundance of major algal groups on periphytometers collected at Refuge transect stations with increasing distance from perimeter canal.

Periphyton taxa indicative of oligotrophic (low P) and eutrophic (high P) conditions in WCA-2A have been identified by DEP based on available literature from the Everglades and other nutrient-impacted systems. Preliminary analyses of periphyton samples collected by the District and DEP along the enrichment gradient in the Refuge indicate that many of the oligotrophic taxa found in interior areas of the Refuge differ from those identified in WCA-2A. Differences in taxonomic composition between these two marshes are attributable to differences in water chemistry: while interior waters of WCA-2A have high concentrations of major ions such as calcium and sodium, waters in the Refuge are depleted in these and most other ions. Water chemistry conditions in

peripheral areas of both marshes are enriched in both P and other major ions. Thus, periphyton taxa indicative of high P conditions tend to be similar in the two marshes. This similarity is illustrated for two of the more common eutrophic indicators, the diatoms *Gomphonema parvulum* and *Nitzschia amphibia* (Figure 3-36). As is common for indicator taxa, neither of these species is found in every periphyton sample regardless of nutrient concentration. However, when present, the abundance of both species increased with increasing P enrichment. Furthermore, the pattern of response was similar between the two marshes. More extensive analyses of periphyton taxonomic changes in the Refuge and their relationship to P enrichment are currently under way.

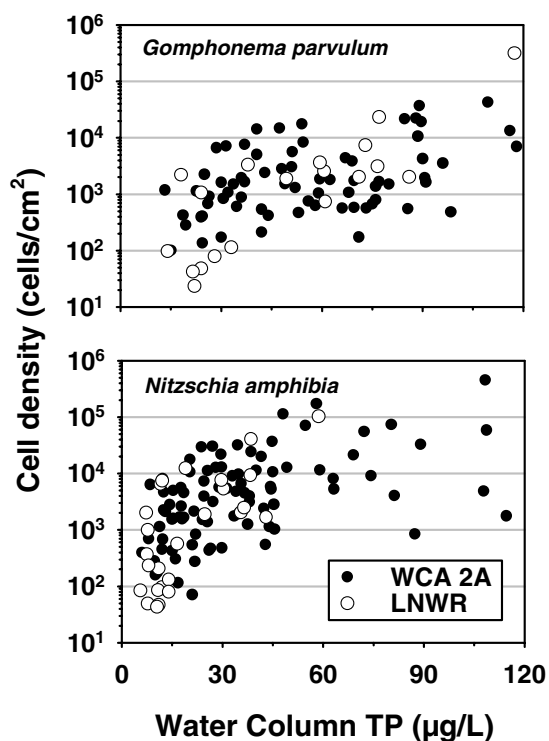


Figure 3-36. Comparison of changes in density of two common eutrophic periphyton indicator taxa as a function of water column TP concentration along nutrient gradients in WCA-2A and Refuge.

Slough Macrophytes

Changes in slough vegetation were documented along the enrichment gradient in the Refuge using ground-based sampling methods. A 50-m transect was established through the middle of a slough adjacent to each permanent transect site. Changes in the abundance of each macrophyte species was tallied in 25 equally spaced 1-m² quadrats along this transect. Three soil samples were collected along each transect, and the 0-10 cm depth increment of each was assayed for TP.

Patterns of macrophyte change along the enrichment gradient in the Refuge were similar to those documented previously in WCA-2A using identical sampling methods (see McCormick et al., 1999) (**Figure 3-37**). The dominant rooted macro-

phyte in sloughs in the marsh interior was *Eleocharis elongata*. As for *Eleocharis cellulosa* in WCA-2A, *E. elongata* declined in abundance with increases in soil TP and was not found at transect stations where TP exceeded 500 mg/kg. Another common slough macrophyte, *Nymphaea odorata*, increased in abundance with increasing soil TP and was most abundant in sloughs where soil TP ranged between approximately 500-700 mg/kg. *Typha* spp. were not present in sloughs in the marsh interior, but were found with increasing frequency

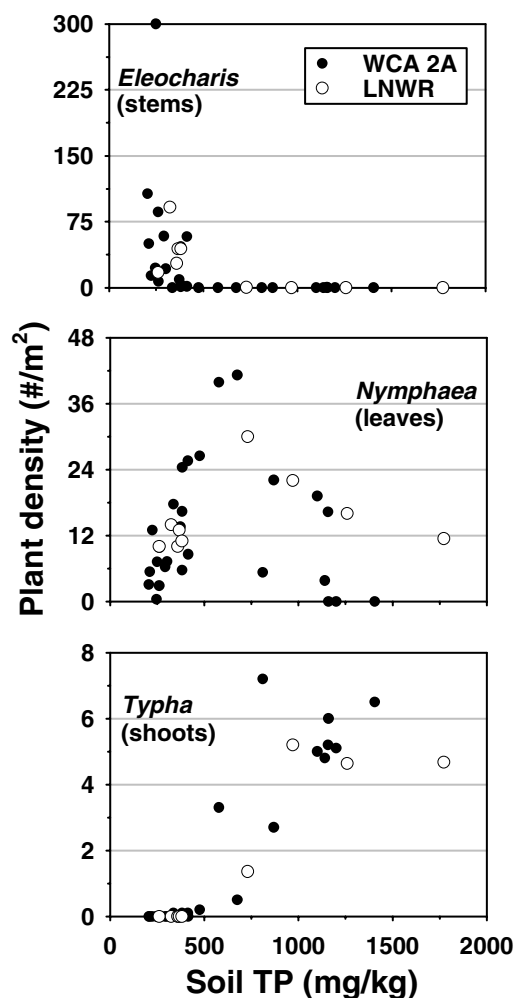


Figure 3-37. Changes in density of three dominant macrophyte taxa as function of soil TP concentration along nutrient gradients in WCA-2A and the Refuge.

in sloughs where soil TP exceeded 500 mg/kg. Experimental field and greenhouse studies (described in McCormick et al., 1999) support the relationship between P enrichment and many of these vegetation changes.

MESOCOSMS

Clear circular enclosures, or mesocosms, similar in design to those used previously by the District in WCA-2A, were installed in a slough in the interior of the Refuge (**Figure 3-17**). Eighteen mesocosms were dosed weekly to achieve one of six annual P loading rates (three replicate mesocosms per load): 0 (controls), 0.2, 0.4, 0.8, 1.6, and 3.2 g P/m²/yr. Doses were prepared and applied in the same manner as for mesocosms in WCA-2A. Dosing was initiated in March 1996 and is expected to continue through the summer of 2000. Changes in chemical and biological conditions are being monitored in the mesocosms and in unenclosed slough plots (outside controls) to detect any changes caused by the enclosures. The following section presents representative changes documented in response to increased P loading during the first two years of dosing.

Soils and Porewater

The effect of increased P loads on Everglades soil chemistry is a slow process. The component that is first and most affected by elevated P loads is the flocculent layer of material resting on the soil surface, which is comprised of unconsolidated plant detritus and/or benthic periphyton. The meso-

cosm experiments conducted in the Refuge (**Figure 3-38**) and WCA-2A (Newman et al., in review) show that the benthic layer lying on the soil surface responds rapidly to P enrichment, while soil P accumulation takes months to years, dependent on the loading rate used (McCormick et al., 1999; Newman et al., in review). A similar accumulation of nutrients in the benthic layer also was observed in a dosing study conducted in the littoral zone marsh of Lake Okeechobee (Havens et al., 1999).

Porewater P concentrations in both the Refuge and WCA-2A mesocosms were slow to respond. After more than two years of P loading in the Refuge, there was no observable increase in either TDP or SRP concentrations in the porewater of any of the treatments. This likely is due to the P limited nature of the marsh and, consequently, the rapid removal of available P by periphyton, bacteria, and macrophytes. In contrast, porewater NH₄-N concentrations in both the Refuge and WCA-2A mesocosms responded rapidly to increased P loads, becoming elevated within four months at higher loads and decreasing over time in the highest loads (Newman et al., in review; **Figure 3-39**). This increase is likely due to increased decomposition and associated N mineralization. Short-term decomposition rates, measured as cotton strip tensile strength loss over time (see transect results above), showed that while there is no apparent increase in soil TP, soil and benthic decomposition rates increase rapidly in response to P addition (**Figure 3-40**).

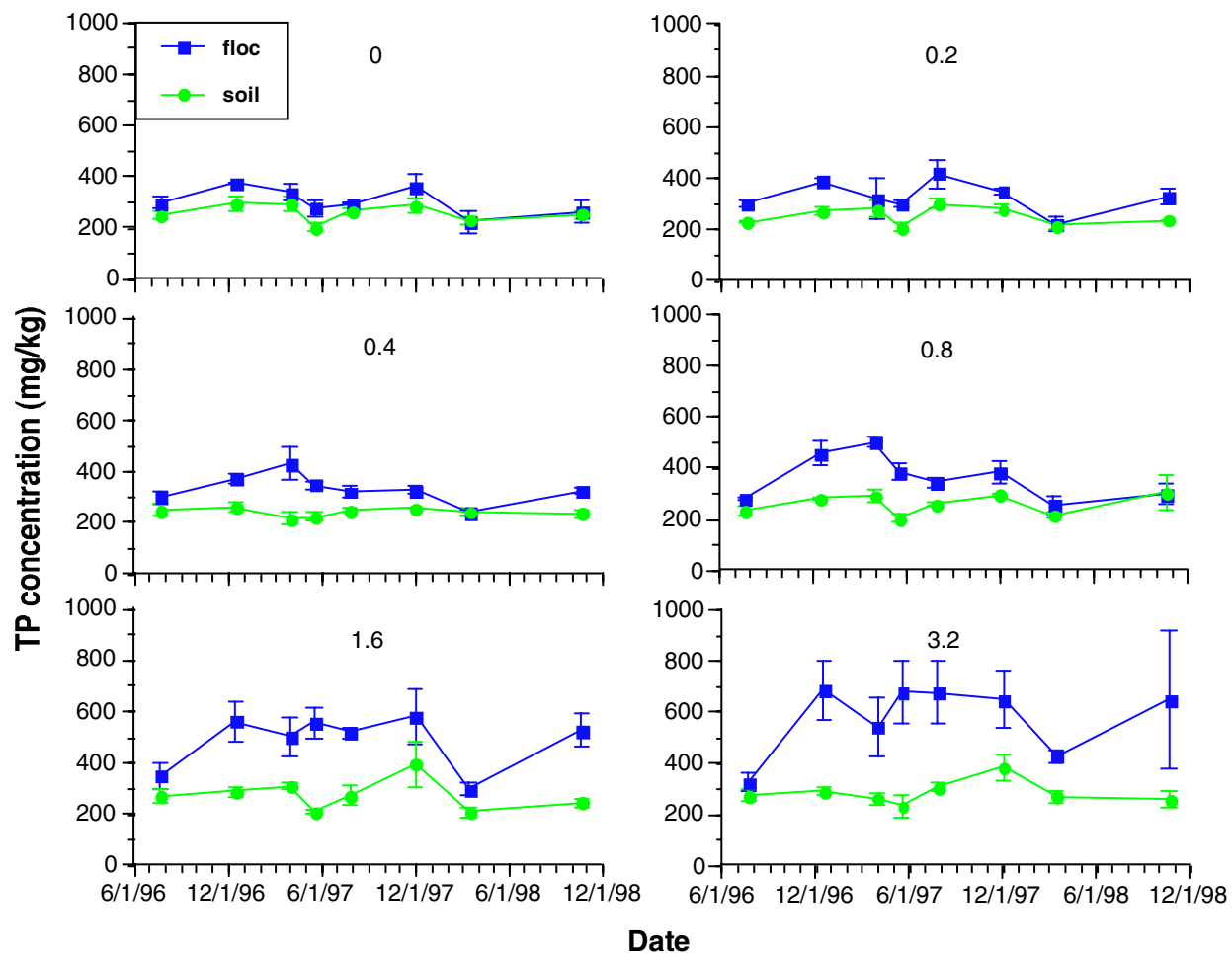


Figure 3-38. TP concentrations (mean \pm SE) in the flocculent layer and surface 0-3 cm soil depth in mesocosms. Soil depth increment was 0-5 cm in December 1996. Numbers at top center of each plot indicate P load applied ($\text{g/m}^2/\text{yr}$).

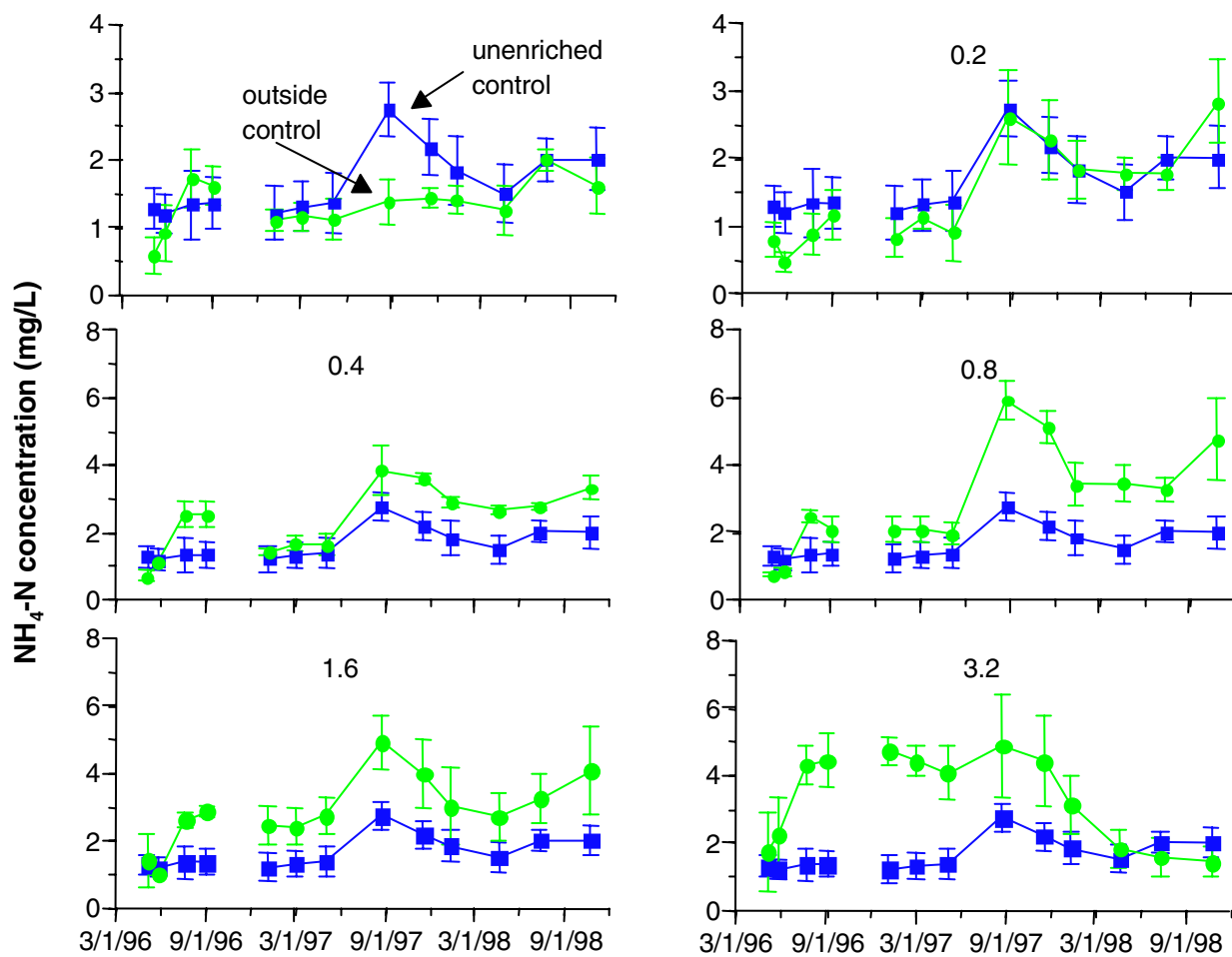


Figure 3-39. $\text{NH}_4\text{-N}$ concentrations (mean ± 1 SE) in the porewater in the mesocosms. Squares = response of unenriched controls, circles = response of P loaded mesocosms. Numbers at top center of each plot indicate P load applied (g/m²/yr).

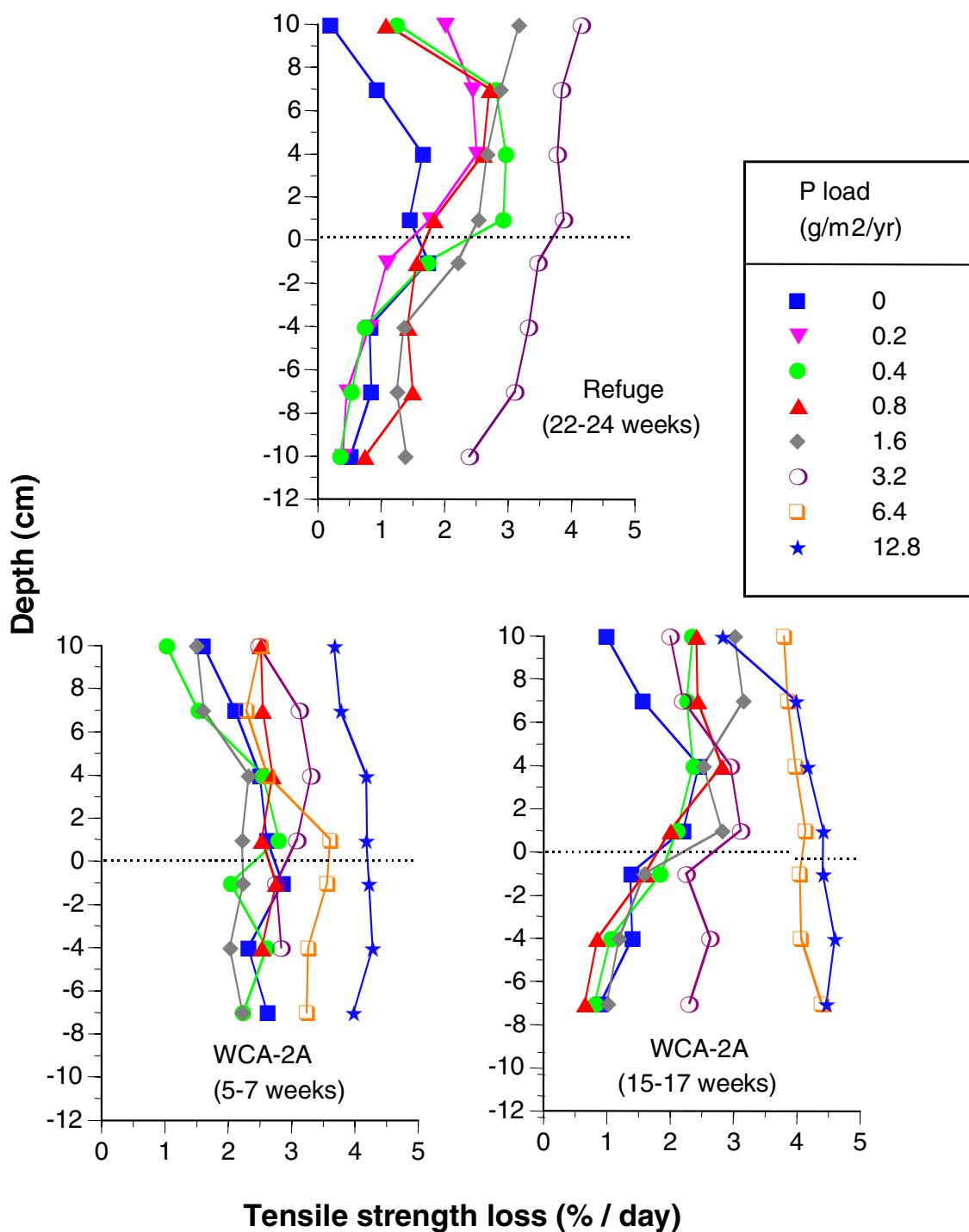


Figure 3-40. Increase in decomposition rates in response to increased P loading in Refuge and WCA-2A mesocosms.

Periphyton Nutrient Content

Periphyton grab samples were collected routinely to measure the nutrient content (carbon [C], N, and P). Sampling of the benthic periphyton layer, or epipelon, was initiated when dosing commenced. The upper 1-2 cm of this layer was sampled using a small dip net. Sampling of the metaphyton, which consisted of periphyton-covered *Utricularia* in unenriched mesocosms and filamentous algae in the higher doses, was initiated late in 1996 (after nine months of dosing). Five grab samples of this material from different locations within a mesocosm were combined into a single sample for nutrient analysis. Samples for nutrient analysis were frozen for later determina-

tion of C, N, and P content, which was reported as concentration per unit dry weight (g/kg).

Epipelon P content was similar in control mesocosms and outside control plots in the surrounding slough during the first two years of dosing (**Figure 3-41**) and ranged between 0.2 and 0.5 g/kg. The P content of this benthic material increased quickly in response to P loading of 1.6 and 3.2 g P/m²/yr and averaged as much as four-fold higher than that in controls during the dry season and between two- to three-fold higher during the wet season. Epipelon P also was consistently higher in mesocosms receiving 0.4 and 0.8 g P/m²/yr compared with controls while little elevation in P was observed at a loading rate of 0.2 g P/m²/yr.

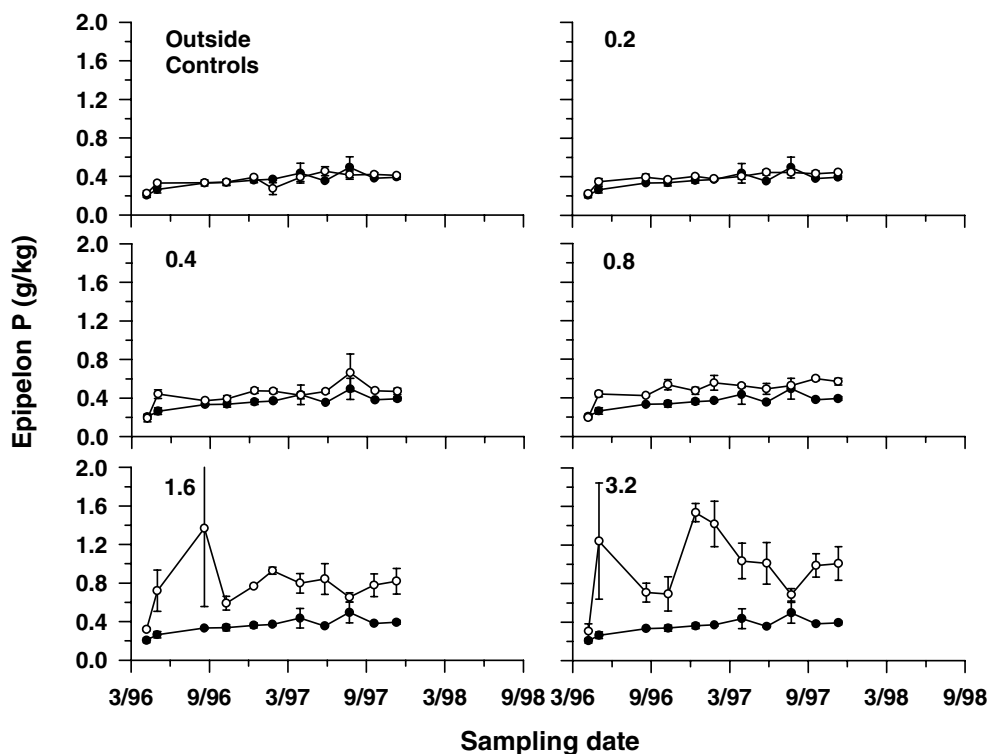


Figure 3-41. Epipelon P content in the Refuge mesocosms. The closed circle in each plot is mean (± 1 SE) value for unenriched mesocosms, while open circle is value for P loaded mesocosms. Numbers in upper left of each plot indicate P load applied (g/m²/yr).

Metaphyton P content also was similar between control mesocosms and outside plots, and ranged between 0.3 and 0.8 g/kg (**Figure 3-42**). Increases in the P content of this assemblage were proportional to the P loading rate and as much as eight-fold higher at a loading rate of 3.2 g P/m²/yr compared with controls. Increases in metaphyton P content were greater than for the epipelon, possibly

due to the higher proportion of actively growing algae and plant material in the floating assemblage and/or a greater exposure to water column P compared with the epipelon. Less seasonality was observed for the P content of the metaphyton, although only one year of data was available at the time of analysis.

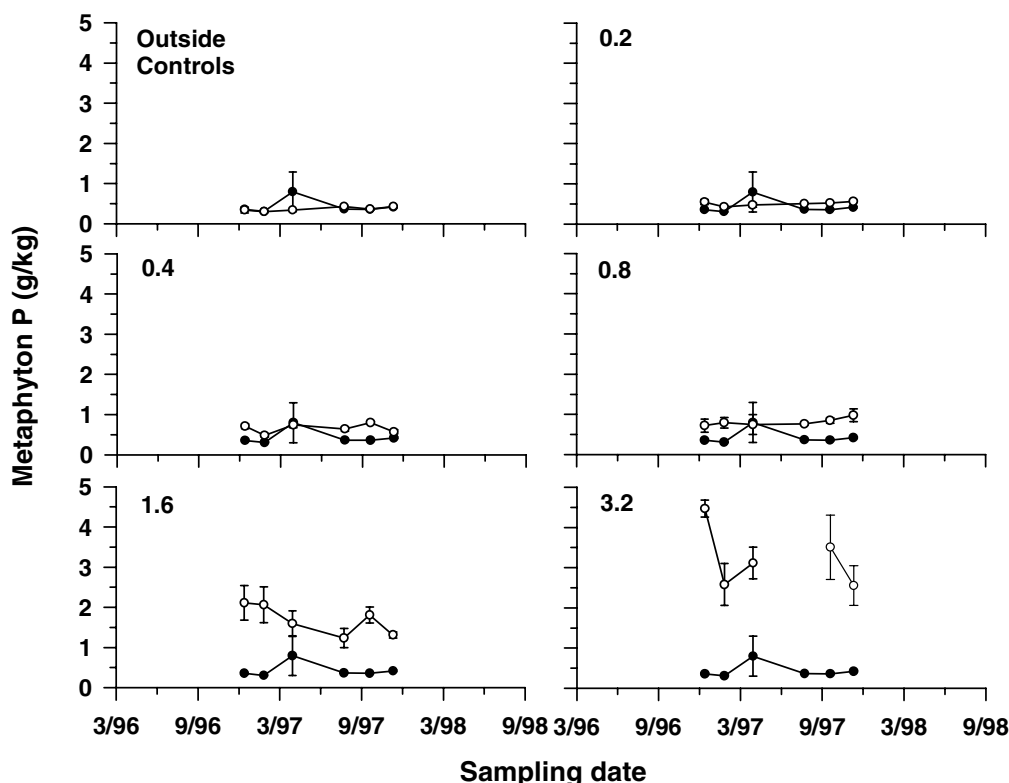


Figure 3-42. Metaphyton P content in Refuge mesocosms. The closed circle in each plot is mean (± 1 SE) value for unenriched mesocosms, while open circle is value for P loaded mesocosms. Numbers in upper left of each plot indicate P load applied (g/m²/yr).

Epipelon and metaphyton N content in control mesocosms and the surrounding slough ranged between 31 and 43 g/kg and 23 and 28 g/kg, respectively (**Figures 3-43 and 3-44**). Epipelon N content was slightly higher (generally <15 percent) in P loaded mesocosms compared with controls. Increases in metaphyton N content were greater

and approached 30-50 percent at the two highest loading rates. Increased periphyton N in enriched treatments may be a result of increased N demand created by increased P supply. Increased N-fixation activity also may be occurring in enriched mesocosms as a result of a shift from P towards N limitation.

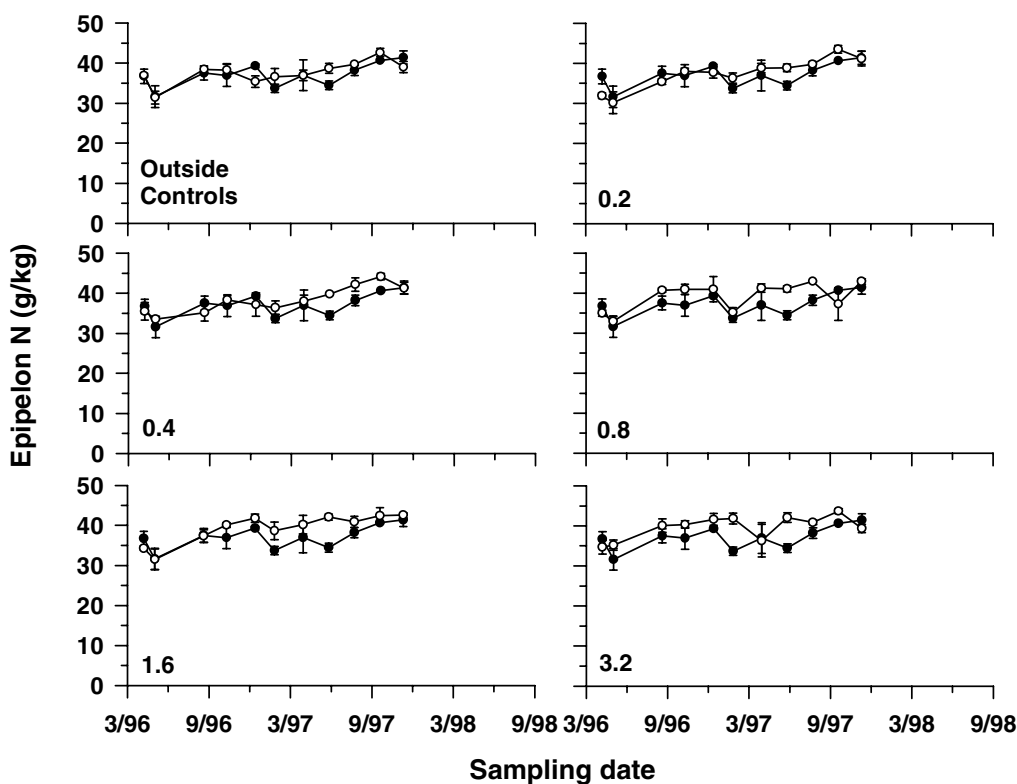


Figure 3-43. Epipelon N content in the Refuge mesocosms. The closed circle in each plot is mean (± 1 SE) value for unenriched mesocosms, while open circle is value for P loaded mesocosms. Numbers in lower left of each plot indicate P load applied (g/m²/yr).

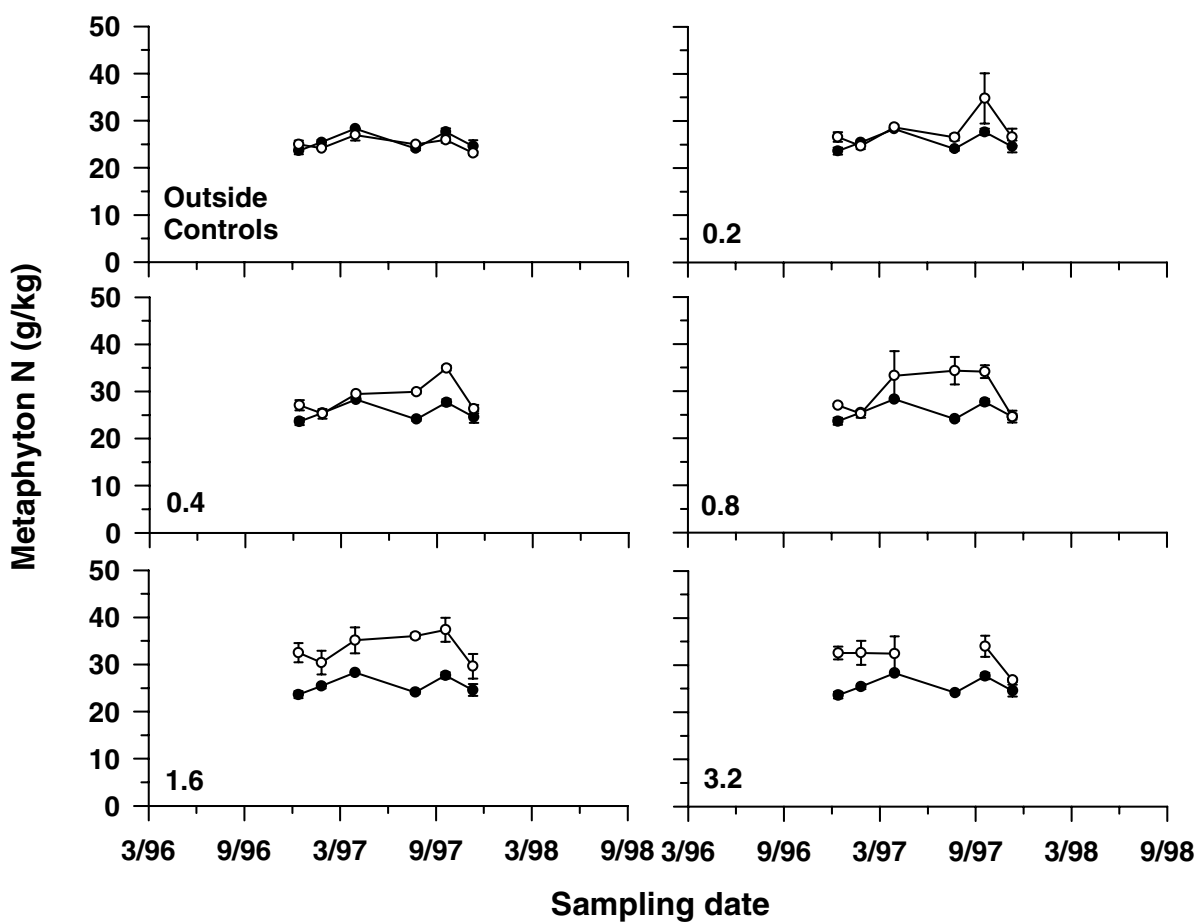


Figure 3-44. Metaphyton N content in the Refuge mesocosms. The closed circle in each plot is mean (± 1 SE) value for unenriched mesocosms, while open circle is value for P loaded mesocosms. Numbers in lower left of each plot indicate the P load applied ($\text{g/m}^2/\text{yr}$).

Temporal patterns of P accumulation by the epipelton in response to P enrichment were similar in the Refuge and in WCA-2A, as illustrated for mesocosms exposed to P loads of 3.2 g/m²/yr (**Figure 3-45**). During the first two years of loading, epipelton TP concentrations in both marshes gradually increased to between 0.8 and 1.2 g/kg, which were roughly two to four times background levels. This accumulation process was relatively steady in WCA-2A, but was punctuated by periods of higher (>1.2 g/kg) P concentrations in the Refuge. Metaphyton P concentrations were considerably higher in Refuge mesocosms receiving 3.2 g P/m²/yr than in comparable mesocosms in WCA-2A after one year of enrichment (**Figure 3-45**). However, P continued to accumulate steadily in the WCA-2A metaphyton during the second year of loading and approached levels in the Refuge assemblage during this period. By the end of the second year, both assemblages had accumulated P to concentrations roughly five to ten times background levels.

Periphyton Metabolism

Rates of periphyton productivity and respiration were measured by collecting samples of metaphyton and incubating them in uncovered ("light") and covered ("dark") biological-oxygen-demand (BOD) bottles to measure oxygen production and/or consumption. These samples were collected in conjunction with sampling for periphyton nutrient content and taxonomy. Metabolism was measured as the amount of oxygen produced in light bottles per unit light received (gross primary productivity (GPP)) and consumed in dark bottles per unit time (respiration). Metabolic rates were corrected for water column activity and normalized for the amount of periphyton biomass (ash-free-dry-mass or AFDM) in each bottle. Methodological details and calculations are described in McCormick et al., 1998.

Rates of periphyton GPP (**Figure 3-46**) and respiration (**Figure 3-47**) were similar in control mesocosms and the surrounding slough and increased rapidly in response to increased P load-

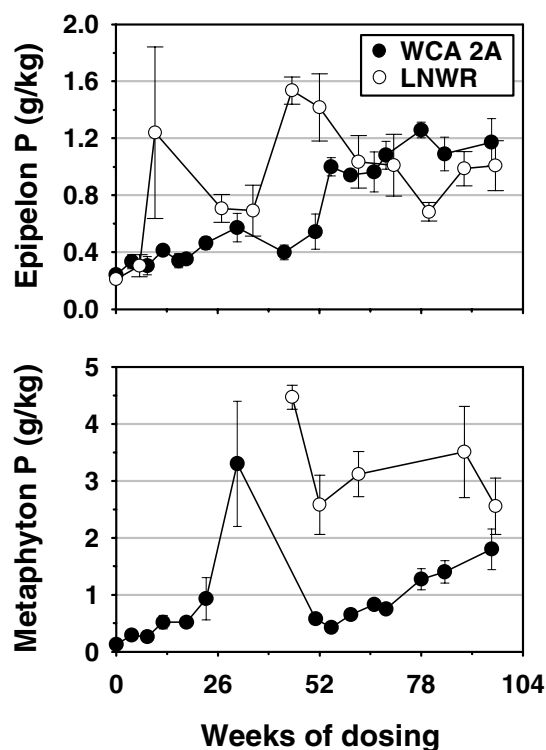


Figure 3-45. Comparison of changes in periphyton P content with duration of dosing in mesocosms exposed to P loading rates of 3.2 g/m²/yr in WCA-2A and Refuge. Numbers in upper left of each plot indicate P load applied (g/m²/yr).

ing. Metabolic rates were seasonal in all loading treatments and the greatest differences between control and P loaded mesocosms occurred during the wet season, when periphyton metabolism was greatest. Metabolic rates were proportional to the P loading rate and, after three years of dosing, there is evidence of a progressive increase in these rates with continued P loading at higher rates (0.8 to 3.2 g P/m²/yr). For example, periphyton exposed to the highest loading rate (3.2 g P/m²/yr) exhibited biomass-specific productivity rates during years one, two, and three of dosing that were 1.7, 3.0, and 3.6 mg O₂/g AFDM/unit light higher, respectively, than unenriched mesocosms. Similar patterns were indicated for periphyton respiration and for other P loading rates, although the strength of these temporal relationships varied.

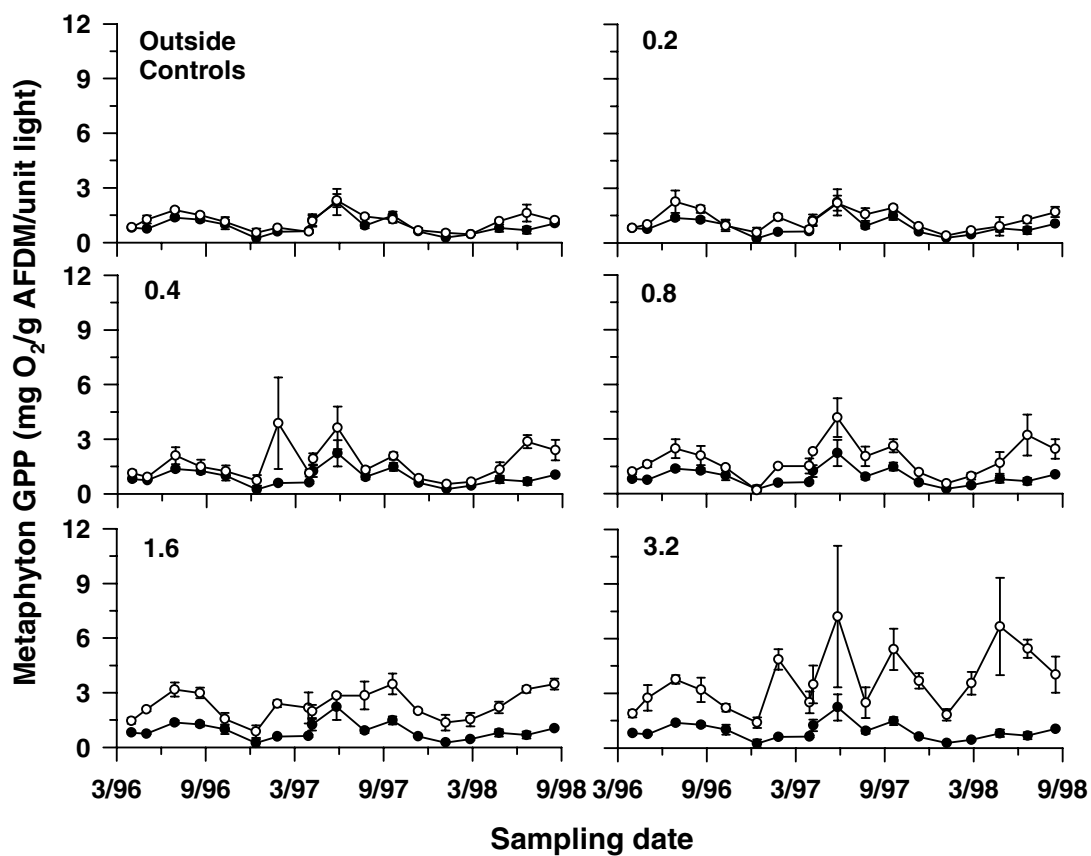


Figure 3-46. Metaphyton GPP in Refuge mesocosms. The closed circle in each plot is mean (± 1 SE) value for the unenriched mesocosms while the open circle is the value for P loaded mesocosms. Numbers in upper left of each plot indicate P load applied (g/m²/yr).

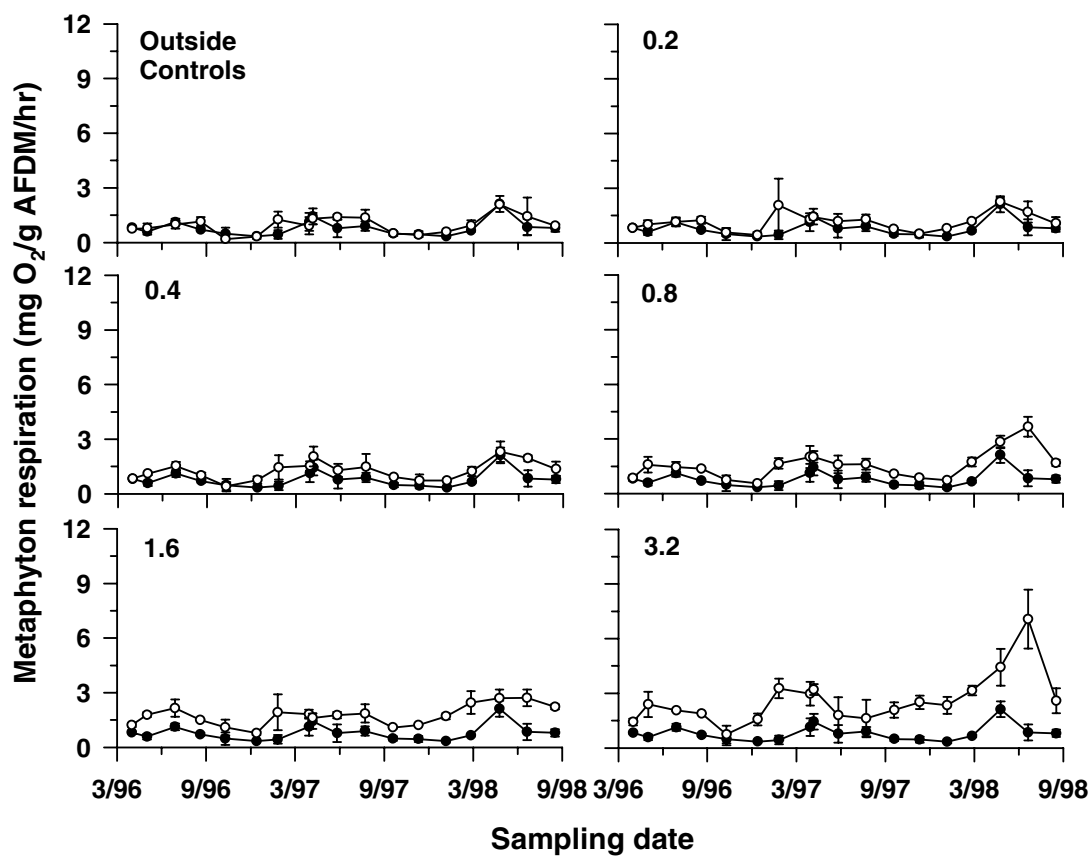


Figure 3-47. Metaphyton respiration in Refuge mesocosms. The closed circle in each plot is mean (± 1 SE) value for unenriched mesocosms, while open circle is value for P loaded mesocosms. Numbers in upper left of each plot indicate P load applied (g/m²/yr).

Increases in periphyton productivity in Refuge mesocosms were similar to those observed in WCA-2A, as illustrated for mesocosms exposed to P loads of 3.2 g/m²/yr (**Figure 3-48**). Metaphyton GPP values generally were two to four times higher than background levels in both sets of mesocosms during the first six months of loading. Following a divergence during the next six months, GPP in both sets of mesocosms continued to oscillate in a similar pattern at rates that were generally two to six times higher than background levels.

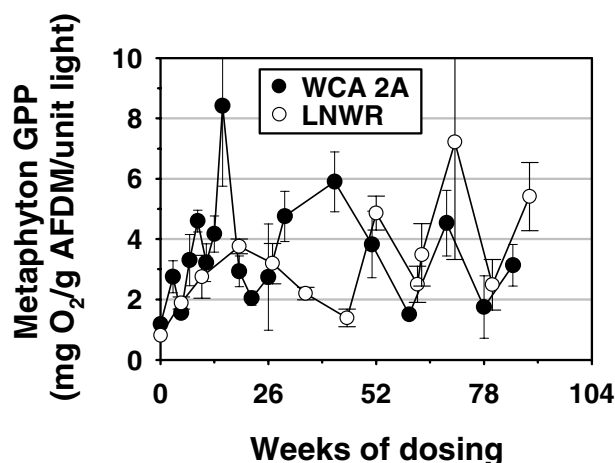


Figure 3-48. Comparison of changes in metaphyton productivity (mean \pm 1 SE) with duration of dosing in Refuge mesocosms exposed to P loading rates of 3.2 g/m²/yr in WCA-2A and the Refuge. Numbers at the top center of each plot indicate P load applied (g/m²/yr).

Macrophyte Nutrient Content

Tissue nutrient concentrations of the two dominant slough macrophytes, *Nymphaea odorata* and *Eleocharis elongata*, increased in response to higher loading rates (**Figure 3-49**). Tissue TP concentrations of both species increased after one year of P loading at rates of 3.2 g/m²/y. After two years of dosing, average TP concentrations of *Eleocharis* were elevated at loads as low as 0.8 g/m²/y, while *Nymphaea* TP content was still elevated only at the highest loading rate of 3.2 g/m²/y.

Increased P loading also appeared to increase the uptake of N (**Figure 3-50**). Average tissue TN concentrations of *Eleocharis* were elevated at a loading rate of 3.2 g/m²/yr after one year of dosing and at all loads after two years. The average TN concentration of *Nymphaea* was elevated only at a loading rate of 3.2 g/m²/yr during the first two years of dosing. Increased utilization of N by both species was dependent upon increases in the availability of P, which is the principle nutrient limiting plant growth.

PRELIMINARY CONCLUSIONS

Data collection by the District in the Refuge is ongoing and, therefore, conclusions based on available information from 1996-1998 should be viewed as preliminary. However, initial findings clearly show that the Refuge is an oligotrophic, P limited marsh that is sensitive to increased P inputs. Many of the chemical and biological changes documented in the Refuge are similar to those documented previously in WCA-2A, and preliminary analyses suggest that the sensitivity of the biota in the two marshes to P enrichment also is similar.

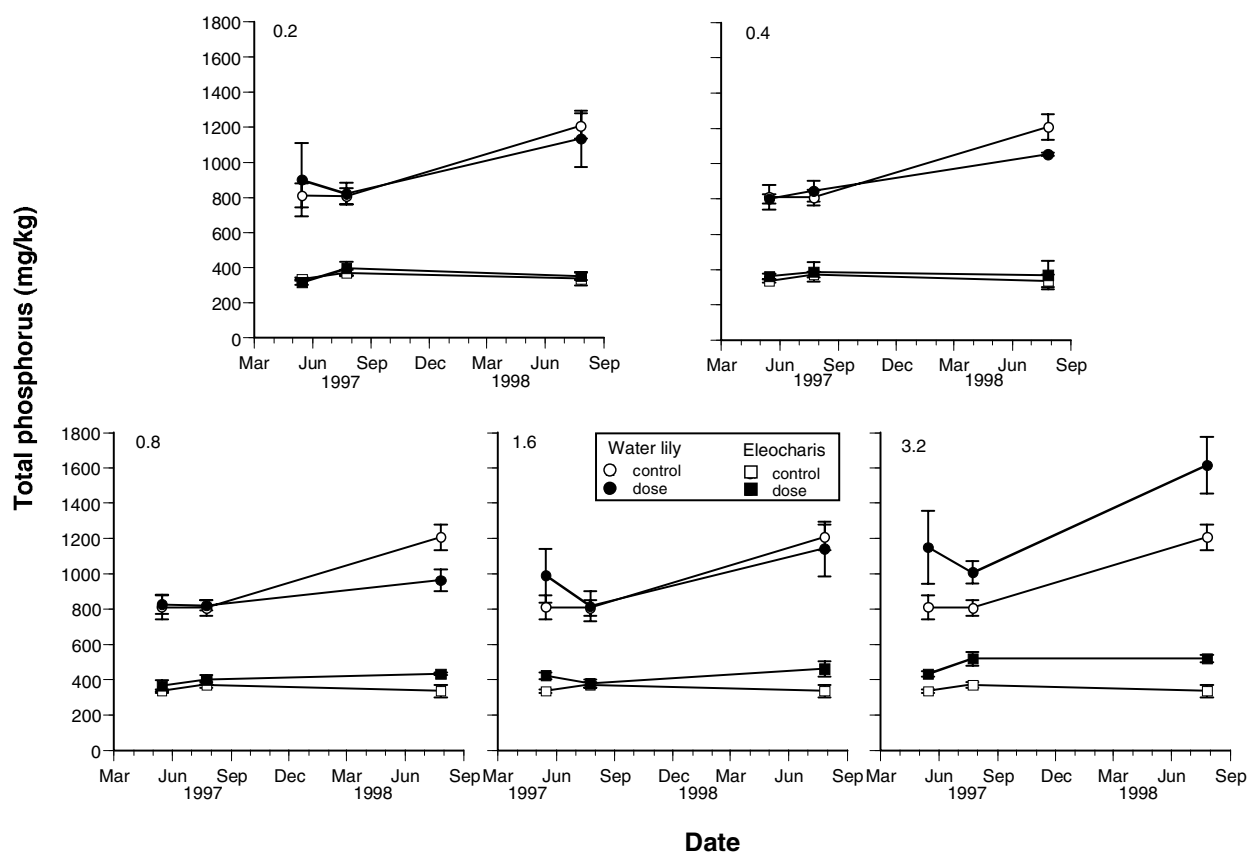


Figure 3-49. *Nymphaea* and *Eleocharis* P content in Refuge mesocosms after one (1997) and two (1998) years of P loading. The open symbol in each plot is mean (± 1 SE) value for unenriched mesocosms, while closed circle is the value for P loaded mesocosms. Numbers in upper left of each plot indicate P load applied (g/m²/yr).

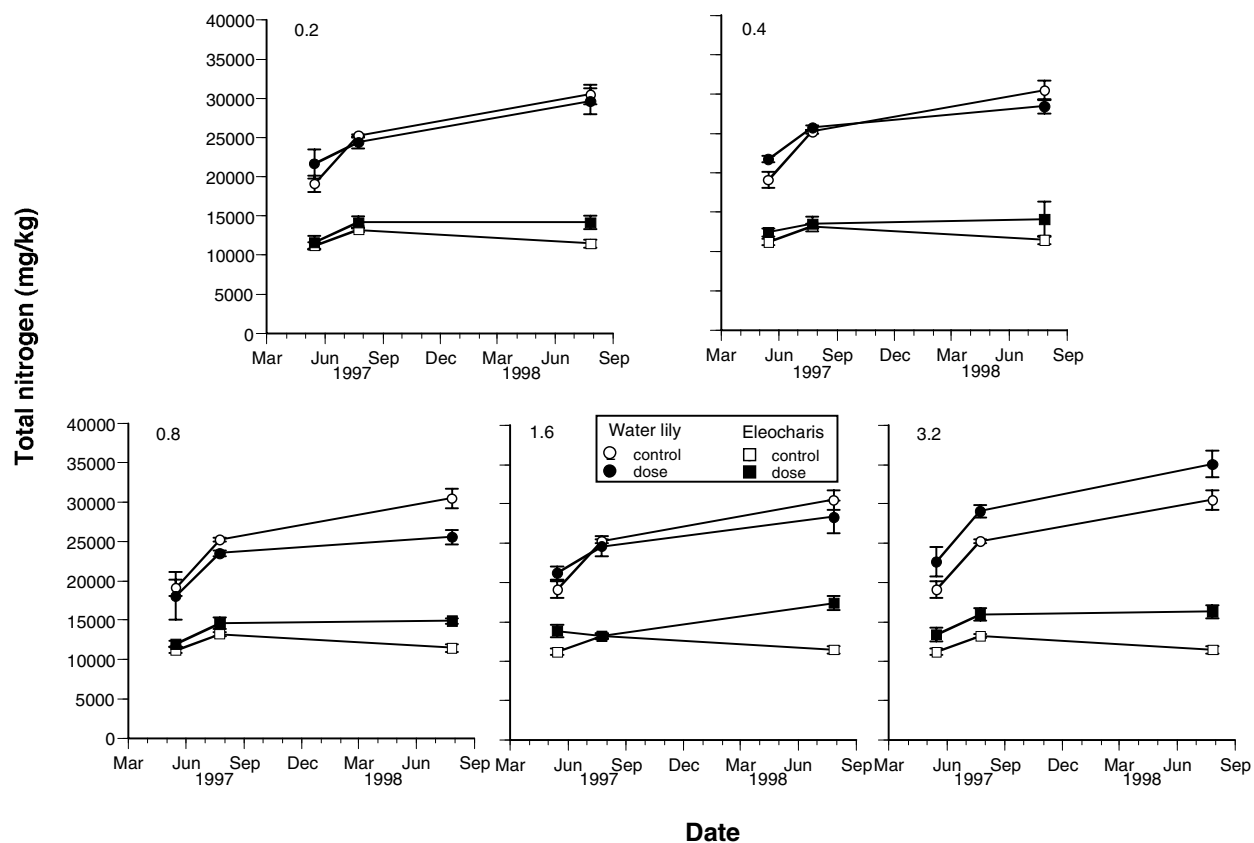


Figure 3-50. *Nymphaea* and *Eleocharis* N content in Refuge mesocosms after one (1997) and two (1998) years of P loading. The open symbol in each plot is the mean (± 1 SE) value for unenriched mesocosms, while closed circle is the value for P loaded mesocosms. Numbers in upper left of each plot indicate P load applied (g/m²/yr).

CENTRAL AND SOUTHERN EVERGLADES

EPA RULING

In May 1999, the EPA approved the 10 µg TP/L column quality standard adopted by the Miccosukee Tribe of Indians of Florida (Tribe, MTIF 1997). The water quality standard applies to Class III-A waters within tribal boundaries, defined by the Tribe as tribal water bodies used for “fishing, frogging, recreation (including airboating), and the propagation and maintenance of a healthy, well-balanced population of fish and other aquatic life and wildlife...primarily designated for preservation of native plants and animals of the natural Everglades ecosystem”. While tribal waters are located within the interior of WCA-3A, which has median background TP concentrations ranging 4-10 µg/L (McCormick et al., 1999), the EPA determined that at present there are no data that suggest P concentrations <10 µg/L cause changes in flora or fauna. Citing peer-reviewed publications and technical reports, the EPA determined that the 10 µg/L standard was a “scientifically defensible value which is not overly protective” and will protect the Class III-A designated use. It also stated, however, that additional Everglades data are still being collected and if further studies show that 10 µg/L is not protective of Class III-A waters, then the Tribe should revise its standard as necessary.

In approving the numeric standard, the EPA recognized that no single study could be used to set the standard but a comprehensive understanding could be obtained using data from numerous Everglades studies. Using a review approach similar to that of McCormick et al., (1999), the EPA first considered reference conditions and subsequently examined P impacts measured in the system.

Reference Conditions

The establishment of reference conditions was the first step in determining how sensitive the tribal lands were to P enrichment. The principal conclu-

sion obtained from Everglades literature was that the Everglades marshes are naturally extremely oligotrophic and unimpacted interior areas have long-term average water column TP concentrations ≤10 µg/L. Plant and animal communities, therefore, both developed and adapted to a low P environment. Field studies and controlled experiments confirmed that P was the primary limiting nutrient. Thus, all the evidence suggested that native plant and animal communities of tribal lands would be adversely impacted by P enrichment.

Impacts of P Enrichment

A key to the EPA determination of impact was the tribal designated use for Class III-A waters. A loss of structure and function of natural Everglades ecosystem was considered contrary to the tribal Class III-A designated use of “preservation of native plants and animals...”. Therefore, the EPA evaluated the effects of P enrichment on various components of the Everglades ecosystem including the response of microbes, periphyton, macrophytes, soil, DO, and wading bird foraging habitat. The specific details of these impacts are described in McCormick et al., (1999). The widely documented sensitivity of native periphyton, macrophyte, and microbial communities to P enrichment was used by EPA to support the Miccosukee’s proposed P standard.

DISTRICT RESEARCH INITIATIVE IN THE CENTRAL-SOUTHERN EVERGLADES

During 1999, the District obtained funding from EPA to conduct gradient and experimental studies in WCA-3A and Taylor Slough (Everglades National Park). A principal focus of this work is to compare the sensitivity of peat (WCA-3A) and marl (Taylor Slough) slough-wet prairie habitats to P enrichment. While considerable attention has focused on responses of Everglades peatlands to P

enrichment, little information currently is available with which to assess effects on marl-forming marshes. Both gradient (transects) sampling and mesocosm dosing experiments will be conducted as part of this central-southern Everglades project. Sampling commenced in August 1999 and will

continue through August 2000, with initial results becoming available during 2000. Results will be compared with those from northern Everglades marshes (WCA-2A, the Refuge) to assess spatial variation in ecosystem sensitivity to P enrichment.

EXPERIMENTS TO UNDERSTAND MECHANISMS UNDERLYING VEGETATION CHANGES IN THE EVERGLADES

Changes in macrophyte abundance and species composition constitute some of the most dramatic responses to P enrichment in the Everglades. These changes can have important ramifications for the abundance and distribution of other native species and the rates of key ecosystem processes (e.g., community metabolism and energy flow, nutrient cycling, soil accretion). Several field and greenhouse studies have been conducted to understand how increased P levels interact with other environmental disturbances (e.g., hydroperiod changes, fire) to cause shifts in Everglades vegetation (reviewed in McCormick et al., 1999). These studies have shown how differences in life history strategies of dominant macrophyte species (e.g., *Cladium* and *Typha*) may affect their response to P enrichment and other environmental changes. Sawgrass plants exhibit slow growth, extended life cycles, low reproductive yield, and an inability to alter biomass allocation (e.g., storage vs. photosynthetic tissues) in response to changes in the resource environment. By contrast, cattail is characterized by high growth rates, a short life cycle, high reproductive output, and other traits that confer a competitive advantage under enriched conditions. Continued experimentation described below has provided additional information on interspecific differences in growth, nutrient uptake, biomass allocation, germination, and seedling growth in response to P availability and on the interactive effects of P enrichment and other environmental changes.

RELATIONSHIP BETWEEN CATTAIL GROWTH AND P

A field reciprocal transplant experiment, in which cattail plants were transplanted to enriched and unenriched sites, showed a close relationship between cattail expansion and P enrichment (Miao et al., submitted). Differences in cattail response between the sites were measured in terms of the relative growth rate (RGR), usually expressed as the amount of biomass produced per unit biomass per unit time. After seven months, plants (per genet) transplanted to the enriched site exhibited greater RGR (22 vs. 8 mg/g/d) and biomass accumulation (152 vs. 14 g) than plants transplanted to the unenriched sites. Each plant transplanted to the enriched site produced an average of six new ramets, covering approximately 1.2 m² of marsh area. In contrast, no ramets were produced at unenriched sites.

DIFFERENTIAL CAPACITY FOR SPATIAL EXPANSION BY SAWGRASS AND CATTAIL

Seedlings of sawgrass and cattail were planted in plastic pots containing soil collected from either a P enriched site or P unenriched site in WCA-2A. Four holes at the bottom of each pot allowed rhizomes (i.e., new roots) and ramets (i.e., new shoots) to spread outside the pot. The pots were placed in outdoor tanks filled with either P enriched (TP=162 µg/L) or P unenriched (TP=10 µg/L) water and allowed to grow for over one year.

Plants of both species rarely produced rhizomes in unenriched pots. Plants of both species produced more rhizomes and total biomass (i.e., dry weight of live tissues) in the enriched treatment than the unenriched treatment. Increases in sawgrass biomass with enrichment exceeded that for cattail. This finding is consistent with previous field measurements (Miao and Sklar, 1998). However, cattail allocated more biomass to rhizomes than sawgrass. While all sawgrass ramets grew close together within the pots, cattail rhizomes and ramets spread widely outside the pots; therefore, one cattail genet occupied a much larger area than one sawgrass genet. These results suggest that although sawgrass plants can grow vigorously under P enriched conditions, they have morphological constraints that limit their spatial expansion. In contrast, the long rhizomes of cattail plants facilitate the rapid occupation of open areas.

PHYSIOLOGICAL RESPONSES TO P ENRICHMENT

Several physiological experiments conducted under contract with the District have involved the use of a PhytoNutriTron (PNT), a computer-controlled hydroponic growth facility at the Department of Plant Ecology, University of Aarhus, Denmark (Lorenzen et al., 1998). The hydroponic rhizotron system consists of four independent growth units each containing eight root vessels built into a controlled growth chamber in a block design. The growth chamber regulates air temperature, humidity, and light intensity. Each of the four growth units are connected to a separate, temperature, pH, and oxygen controlled reservoir (180 L) through which the nutrient solution is recirculated. The reservoirs are equipped with UV-sterilization units, and the concentrations of ammonium N and phosphate are monitored continuously through an auto-analyzer using standard colorimetric methods. Nutrient concentrations are maintained at constant levels through computer-mediated feedback regulation, which supplies nutrients at rates equivalent to their depletion. This constant nutrient supply maintains a known P concentration at the root sur-

face. Under field conditions, by contrast, P concentrations at the root surface are less than those in the porewater as a result of plant uptake. Therefore, laboratory P conditions are not directly comparable to those in the field.

Experiments investigated the effects of P availability on the RGR, biomass allocation and nutrient use efficiency in sawgrass and cattail (Lorenzen et al., in review). Sawgrass and cattail responded differently to changes in P availability. The RGR of cattail was more than twice as high as that of sawgrass at all the steady state P levels. No clear relationship existed between the P levels and the RGR of sawgrass, which ranged between 19 to 37 mg/g/d at the four steady state P concentrations (10, 40, 80, and 500 μg SRP/L). Similarly, P levels had little effect on growth, biomass allocation, and nutrient use efficiency. However, at P concentrations close to C_{\min} , sawgrass was able to maintain a RGR of 10 mg/g/d. In contrast, the RGR of cattail increased from 48 to 89 mg/g/d with increasing steady state P availability. However, cattail was not able to maintain the expected growth rate at C_{\min} and exhibited imbalances in biomass allocation.

Preliminary P uptake kinetics results showed that the two species have similar C_{\min} (2.1 μg P/L). At this concentration, no net uptake of P from culture solutions was measured for either species. Based on plant P accumulation measurements, V_{\max} (the maximum uptake rate) and K_m (the half saturation constant of the Michaelis-Menten equation) were 8 mg P/g root dry wt/d and >80 μg P/L, respectively, for cattail, and 0.5 mg P/g root dry wt/d and 10–40 μg P/L, respectively, for sawgrass. These uptake kinetic parameters show the relative abilities of sawgrass and cattail to utilize P concentrations and hence provide valuable information for determining P threshold values. These parameters are presently being confirmed with more detailed uptake kinetic experiments.

Sawgrass biomass allocation was similar among P treatments while the biomass allocation of cattail to tissues with different functions varied with P availability. This result is consistent with

those from previous field studies (Miao and Sklar, 1998). At high P availability, cattail biomass was largely allocated to leaves, which increased the surface area available for photosynthesis. While at low and intermediate P levels, biomass was allocated to rhizomes (increase the area that the plant can search for nutrients), ramets, and roots (increase the nutrient absorbing surface area of the plant). At a P concentration close to C_{min} , most of the leaf biomass was shifted to the roots, a process that ultimately limited photosynthesis and resulted in plant death.

These results showed that cattail exhibited a high degree of plasticity to P availability, while sawgrass exhibited little response to P availability and had characteristics similar to plants adapted to nutrient poor environments. The results indicated a lower uptake capacity and a lower half saturation constant (K_m) for P accumulation by sawgrass compared with cattail. This finding is consistent with the observed growth responses of both species to P enrichment. Cattail showed a greater RGR than sawgrass at all four steady state P concentrations (10-500 $\mu\text{g P/L}$), suggesting that cattail is the better competitor within this concentration range. However, at the C_{min} (around 2.1 $\mu\text{g P/L}$), sawgrass maintained the expected RGR (13 mg/g/d) while cattail could not. Thus, sawgrass probably has a competitive advantage over cattail at very low P concentrations.

EFFECTS OF P LOADING ON SEED GERMINATION AND SEEDLING GROWTH IN REFUGE MESOCOSMS

Field gradient studies suggest that cattail invasion of a new site is strongly influenced by P availability. To test this hypothesis, a seed germination

and seedling growth experiment was conducted in the Refuge mesocosms. Small plastic pots were filled with soil collected from a nearby slough. Seeds of cattail and sawgrass were spread on the soil surface, and pots were secured in floating racks in each mesocosm to maintain saturated soil conditions. Increased P loading had no apparent effect on seed germination of either species. Cattail germination peaked within 21 days at all loading rates and germination success varied between 3 and 15 percent with no relation to the loading rate. Sawgrass germination occurred over a much longer time period, peaking after six months. Sawgrass germination success ranged between 10 to 25 percent and also was unrelated to the P loading rate.

Seedling survivorship and growth differed between species and was affected by the P loading rate. Cattail survivorship and growth were both increased at higher P loading rates, and no seedlings survived at loading rates $<0.8 \text{ g P/m}^2/\text{yr}$. By the end of the experiment, total live biomass was similar at P loads of 0.8 and 1.6 $\text{g P/m}^2/\text{yr}$ and substantially higher at a load of 3.2 $\text{g P/m}^2/\text{yr}$ (**Figure 3-51**). Phosphorus enrichment had no effect on sawgrass survivorship as few seedlings died following germination at any load. However, as for cattail, increased P loading enhanced seedling growth rates as indicated by an increase in total biomass (**Figure 3-51**).

These findings indicate that P availability is a critical factor affecting cattail growth and survival. These findings are consistent with those of a previous study (Miao et al., in review), which indicated that a viable seed source, P availability, and appropriate water depths are all fundamental factors determining the success of cattail establishment.

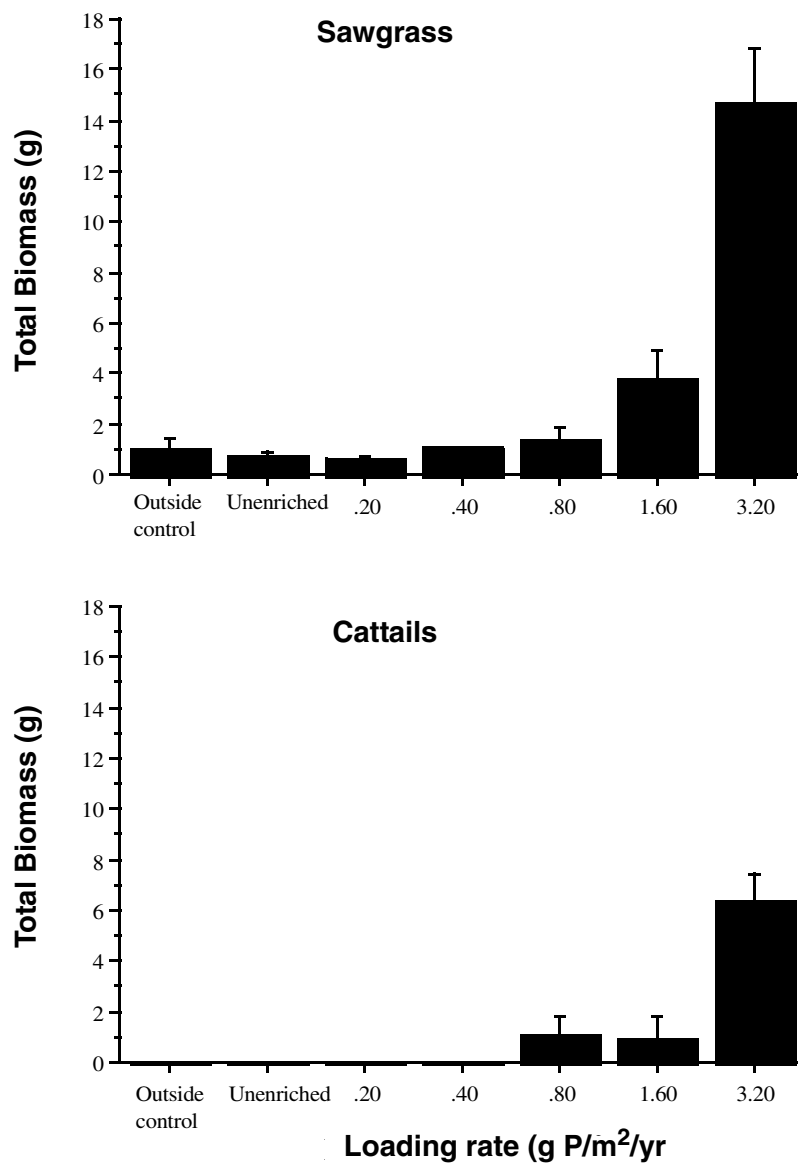


Figure 3-51. Effects of P loading rates on biomass of cattail and sawgrass seedlings grown in Refuge mesocosms for approximately one year. Each bar is mean (± 1 SE) of measurements from three replicate mesocosms.

EFFECTS OF P AND WATER DEPTH ON REGROWTH RATES FOLLOWING FIRE

Ongoing experimentation is investigating the interactive effects of water depth and P concentrations in determining the competitive abilities of sawgrass and cattail. During the last year, an experiment was completed that quantified the effect of these two factors on the recovery of sawgrass and cattail stands after disturbances such as fire that removed above-ground biomass (Miao, unpublished data). Leaves were removed from sawgrass and cattail plants just above the leaf meristem (growing point). Plants were placed in baths containing either P enriched (160 µg/L) or unenriched (10 µg/L) water at depths of either 40 cm or 0-5 cm above the soil surface. One month following leaf removal, leaf regrowth (number of leaves and leaf length), particularly that of sawgrass plants, was greatly inhibited by water depth, whereas no strong P effect was detected. For example, no sawgrass plants recovered after leaf removal at the 40-cm water depth, compared with 30 percent recovery for cattail. These findings suggest that water depth is more important than P concentrations in determining the rate of recovery of established vegetation stands following surface fires. Furthermore, deep water may inhibit the recovery of sawgrass stands more than cattail. Continued experimentation in this area will provide additional information with which to predict the rate of vegetation recovery and the potential for species shifts following disturbance under different hydrologic and P regimes.

CONCLUSIONS FROM EXPERIMENTAL STUDIES

In summary, field studies (Miao and Sklar, 1998; Miao and DeBusk, 1999; McCormick et al., 1999) have demonstrated that P enrichment in WCA 2A has profoundly affected macrophyte physiology (net photosynthetic rates, stomatal conductance, and tissue P concentration), population biology (density, mean plant size, and seed production), and community ecology (species composition, standing biomass, and nutrient accumulation). These alterations in macrophyte communities in turn affect important ecosystem processes such as primary production, nutrient cycling, peat accumulation, and evapotranspiration. Greenhouse and other experimental studies described here support the hypothesis that P is a primary factor limiting the growth and distribution of macrophytes in the Everglades. Sawgrass and cattail exhibit contrasting characteristics throughout their life cycles from seeds, seedlings, young plants, to mature plants that determine their response to P enrichment. Sawgrass is similar to species adapted to low P habitats, whereas cattail is similar to those adapted to high P environments. Experimental studies, such as those described here, have revealed the mechanisms whereby cattail replaces sawgrass under P enriched conditions. This information is valuable not only for understanding P effects in the Everglades, but for devising restoration strategies as well.

CONTINUING RESEARCH EFFORTS

Research and monitoring efforts by the District have expanded to include all major regions of the Everglades. While this work continues to focus principally on open-water (wet prairie, slough) habitats, available information indicates that these ecologically critical areas are especially sensitive

to P enrichment. Therefore, numeric P standards that are protective of these habitats should be protective of the entire marsh. Continued data collection and analyses by other research groups working in specific regions of the Everglades will contribute additional information on the effects of P

enrichment in different regions and habitats. A synthesis of this information has been initiated by DEP to arrive at a numeric P criterion for the Everglades based on best-available scientific information.

New and continuing research activities described in this report are addressing several of the data gaps discussed in the 1999 EFA report (McCormick et al., 1999) including spatial and temporal variation in sensitivity to P enrichment and the interactive effects of P enrichment and

other environmental variables on the ecology of the Everglades. As DEP acquires sufficient data on P effects in different regions of the Everglades, research efforts will need to focus more on the process of marsh recovery following reductions in P loads and concentrations. A better understanding of the expected rate and trajectory of marsh recovery and the ecological processes controlling recovery is critical for directing future management efforts and for setting realistic restoration timelines.

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